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Cortisol secretion patterns in the elderly: in the perspectives of frailty and cognitive function and sleep disturbances as risk factors of cognitive decline

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Summary

The maintenance of good health for both body and mind is important for successful aging in older individuals. The occurrence of multiple chronic conditions in the same individual causes decrements in functional abilities as well as social and psychologic problems that may have an impact on many facets of wellbeing and quality of life in the elderly. Among major contributing factors to decline in an elderly individual's health which are common but still poorly understood are frailty and cognitive impairment.

Dysregulated hypothalamic-pituitary-adrenal (HPA) axis and, in particular, impaired diurnal cortisol secretion is hypothesized as one of the underlying mechanisms for frailty and cognitive decline among the elderly. The hypothalamic-pituitary-adrenocortical (HPA) axis is a critical adaptive system that maximizes energy mobilization for survival potential in the face of physical or psychological challenge. The adrenal hormone cortisol, the end-effector of the HPA axis response, acts on multiple organ systems to maintain homeostatic balance. While short-term exposure to cortisol is beneficial for survival, chronic activation of physiological stress responses may lead to serious metabolic, immune, and psychological dysfunction. There is growing interest in research relating to the role of specific cortisol circadian rhythmicity in influencing various features of health. It is not only the level of circulating cortisol, i.e. either hyper- or hyposecretion may induce the onset of diverse pathological conditions by disrupting carbohydrate and lipid metabolism, immune response, cardiovascular activity, mood and cognitive function, but also its rhythmic activity over the course of a day that plays a significant role in human health and disease. However, to date, there is limited epidemiologic evidence of the impact of specific cortisol patterns in the elderly. Furthermore, cortisol levels are thought to be different in the elderly, due to physiological changes especially in the neuroendocrine system of aged individuals. Thus, further research to examine the impact of specific cortisol secretion patterns on health in older age is warranted.

This thesis comprises three publications which are based on data from the population-based KORA-Age study. The first publication assessed the associations of frailty phenotype and diurnal cortisol secretion pattern in the elderly. Frailty was strongly associated with lower ratios of morning to evening cortisol levels. Participants with slow gait speed had increased evening cortisol levels whereas participants with low grip strength showed lower morning levels.

Additionally, a lower morning to evening ratio was associated with an increased risk of low grip strength and gait speed in the total sample population. Increases in evening cortisol were associated with an increased risk of being categorized in the pre-frail status.

The second publication assessed the association of cognitive function and salivary cortisol secretion patterns in the aged population. Participants with probable dementia and mild cognitive impairment (MCI) had lower means of morning after waking (M1) and 30 minutes after waking (M2) salivary cortisol levels, and higher late evening cortisol levels compared to participants with normal cognition. However, sex-specific significant associations of lower M1 to E, or M2 to E ratios with increased risk for cognitive impairment were observed among men but not women.

The third publication investigated whether sleep disturbances predict cognitive decline in the elderly. Cognitive decline was more pronounced in individuals with difficulties maintaining sleep (DMS) compared to individuals with no DMS. However, the predictive power of DMS was only significant in individuals with normal cognition and not in cognitively impaired subjects at baseline. Prolonged sleep duration increased the risk for cognitive decline in cognitively impaired individuals. Other sleep characteristics of difficulties initiating sleep (DIS) and daytime sleepiness (DS) were not significantly associated with cognitive decline.

In summary, this doctoral thesis provides population-based evidence that dysregulated cortisol secretion patterns are central to frailty and cognitive impairment in the elderly, even when controlling for known confounders of these disabling outcomes. Sleep disturbances have been identified as a potential risk factor of cognitive decline which may offer intervention strategies to deter cognitive decline in the elderly with normal cognitive function. Given the relevance of cortisol as a stress response hormone in impaired sleep and cognitive decline, thus enforces the importance of assisting middle-age and older individuals in stress reduction which is a potentially modifiable risk factor.

Zusammenfassung

Der Erhalt guter körperlicher und geistiger Gesundheit ist wichtig für erfolgreiches Altern. Das Vorkommen von multiplenchronischen Kranheitszuständen in der gleichen Person verursacht sowohl Defizite in der Funktionsfähigkeit als auch soziale und psychologischen Problemen, die sich auf viele Facetten von Wohlsein und Lebensqualität bei älteren Menschen auswirken können. Gebrechlichkeit und kognitive Einschränkung tragen einen großen Teil zu der Verschlechterung der Gesundheit bei älteren Menschen bei; trotz ihrer Verbreitung ist aber nur wenig über sie bekannt.

Das Nebennierenhormon Cortisol unterliegt dem Regelkreis der HNN-Achse. Es wirkt auf mehrere Organssyteme, um das homöostastische Gleichgewicht aufrechtzuerhalten: Mehrere Rückkopplungsmechanismen sorgen für eine homöostatische Regulierung der physiologischen Reaktionskaskade auf einen Stressor und sorgen für eine sinnvolle Anpassung an aktuelle Umweltbedingungen. Während bei akutem Stress ein deutlicher Anstieg des Cortisols erfolgt, kann eine längerfristige chronische Belastung sowohl eine Habituation mit verminderter Ausschüttung von Cortisol über eine physiologische Veränderung der HHN-Achse als auch eine anhaltend ansteigende Ausschüttung bewirken. Die klinischen Folgen einer solchen chronischen Belastung und dauerhaften stress-bedingten Aktivierung des Organismus sind von dem Ausmaß der Cortisolaus-schüttung abhängig und können zu ernsthaften Stoffwechsel-, Immun- und psychologischen Störungen führen. Mit wachsendem Interesse widmet sich die aktuelle Forschung der Frage, welche Rolle bestimmte Cortisol-Tagesrhythmen in unterschiedlichen Gesundheitsbereichen spielen.

Zum einen können pathologische Veränderungen des zirkulierenden Cortisols, d.h. Hyper- oder Hypocortisolismus, durch die damit verbundene Störung des Kohlenhydrat- und Lipidstoffwechsel, der Immunantwort, der kardiovaskuläre Aktivität, der Stimmung oder der kognitiven Funktionen den Ausbruch diversere Krankheitsbilder begünstigen. Zum anderen spielt auch die rhythmische Cortisolaktivität im Laufe eines Tages eine bedeutsame Rolle in der menschlichen Gesundheit.

Dennoch gibt es bisher nur wenige epidemiologische Beweise für die Wirkung spezifischer Cortisolsstruktur bei älteren Menschen. Des weiteren geht man davon aus, dass die

Cortisolspiegel bei älteren Menschen aufgrund altersbedingter physiologischer Veränderungen des neuroendokrinen Systems anders sind. Deshalb sind weitere Forschungsarbeiten nötig, um die Wirkung spezifischer Cortisol-Sekretion-Struktur auf die Gesundheit älterer Menschen zu untersuchen.

Diese Dissertation besteht aus drei Veröffentlichungen, die auf Daten der bevölkerungsbasierten KORA-Age-Studie beruhen. Die erste Veröffentlichung wertete den Zusammenhang zwischen Gebrechlichkeit-Phänotyp und Cortisol-Tagesprofil bei älteren Menschen aus. Gebrechlichkeit war stark mit einem niedrigeren Verhältnis des Morgen- zum Abendcortisolspiegels assoziiert. Teilnehmer mit langsamer Ganggeschwindigkeit hatten erhöhte Abend-Cortisolniveaus, während Teilnehmer mit geringer Griffstärke ein niedrigeres Morgenniveau zeigten. Zusätzlich war ein niedrigeres Morgen-zu-Abend-Verhältnis mit einem erhöhten Risiko für geringe Griffstärke und Ganggeschwindigkeit in der gesamten Stichprobenbevölkerung verbunden. Erhöhungen im Abendcortisol waren mit einem erhöhten Risiko verbunden, als „pre-frail“ (Vorstufe zur Gebrechlichkeit) klassifiziert zu werden.

Die zweite Veröffentlichung wertete den Zusammenhang kognitiver Funktion und Speichelcortisol-Sekretion-Strukturen in den höheren Altersgruppen aus. Teilnehmer mit wahrscheinlicher Demenz oder leichte kognitive Beeinträchtigung (LKB) hatte niedrigere Mittelwerte von Speichelcortisolspiegeln am Morgen nach dem Aufwachen (M1) und 30 Minuten nach dem Aufwachen (M2) sowie höhere Später-Abend-Cortisolspiegl als Teilnehmer mit normaler Kognition. Signifikante Zusammenhänge von niedrigen M1-zu-E oder M2-zu-E-Verhältnissen mit erhöhtem Risiko für kognitive Einschränkung wurden nur bei Männernbeobachtet, nicht aber bei Frauen.

Die dritte Veröffentlichung untersuchte, ob Schlafstörungen kognitive Verschlechterung bei älteren Menschen prognostizieren. Kognitive Verschlechterung trat häufiger bei Personen mit Durchschlafstörungen (DMS) als bei Personen ohne DMS auf. Dennoch war die Vorhersagekraft von DMS nur signifikant bei Personen mit normaler Kognition zur Baseline und nicht bei Personen mit beeinträchtigter Kognition zur Baseline. Lange Schlafdauer erhöhte das Risiko für kognitive Verschlechterung bei kognitivbeeinträchtigten Personen. Einschlafstörungen (DIS) und Tagesschläfrigkeit (DS) waren nicht signifikant mit kognitiver Verschlechterung verbunden.

Zusammenfassend wird in dieser Doktorarbeit anhand bevölkerungsbasierter Daten gezeigt, dass gestörte Cortisol-Secretion-Muster erheblich zu Gebrechlichkeit und kognitiver Verschlechterung bei älteren Menschen beitragen, sogar nach der Kontrolle für bekannte charakteristische Störfaktoren. Schlafstörungen wurden bereits als mögliche Risikofaktoren für kognitiver Verschlechterung identifiziert; Interventionsstrategie können hier ansetzen, um die kognitive Verschlechterung in älteren Menschen mit normaler kognitiver Funktion abzuhalten. Angesichts der Relevanz des Cortisols als ein Stressantwort-Hormon bei gestörtem Schlaf und kognitiver Verschlechterung ist es wichtig, Menschen im mittleren und fortgeschrittenen Alter Strategien zum Stressabbau an die Hand zu geben, da Stress ein potenziell veränderbarer Risikofaktor ist.

1. Introduction

1.1 Hypothalamic-pituitary-adrenal (HPA) axis: cortisol synthesis

Cortisol, the main glucocorticoid (GC) in man is an end-effector of the hypothalamic-pituitary-adrenal (HPA) axis activation (**Figure 1**). The principal effectors of the stress response are localized in the paraventricular nucleus (PVN) of the hypothalamus, the anterior lobe of the pituitary gland, and the adrenal gland as depicted in **Figure 2**. This collection of structures is commonly referred to as the HPA axis. In reaction to both physical and psychological stress, corticotrophin-releasing hormone (CRH) is released from the paraventricular nucleus (PVN) of the hypothalamus [1]. Consequently, CRH binds on the type 1 corticotrophin releasing hormone receptors (CRH-R1) on the anterior pituitary corticotoph cells stimulating the release of adrenocorticotropic hormone (ACTH) from the pituitary gland to the peripheral circulation [2]. In turn, ACTH binds to the type 2 melanocortin receptor (MC2-R) in the zona fasciculate cells of the adrenal cortex, resulting in the secretion of the steroid hormone cortisol which interacts with specific receptors in various target tissues in the brain and periphery [3]. Circulating GC ultimately turns off the HPA axis activity and restores a steady state via negative feedback mechanisms.

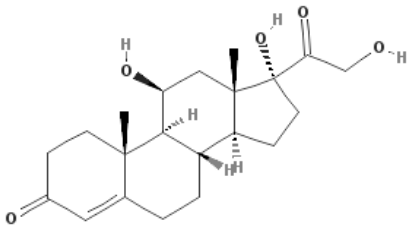

Figure 1. Structure of Cortisol [4]

1.2 Cortisol: mechanism and action of the HPA axis

The HPA axis is a critical adaptive system that maximizes energy mobilization for survival potential in the face of physical or psychological challenge [5]. As an end-effector of the stress-responsive HPA axis, cortisol exerts widespread effects on multiple organ systems to maintain homeostatic balance and enable the organism to respond to and cope with physical and emotional stressors, aimed primarily at metabolic functioning by increasing energy availability in target tissues [6] as well as affecting the immune response [7], and even memory [8] .

Besides that, physiological adaptations initiated by activation of this system include increased cardiovascular tone, respiratory rate, and intermediate metabolism, along with inhibition of general vegetative functions such as feeding, digestion, growth, reproduction, and immunity [6, 9]. In addition to the HPA axis, several other anatomic structures play important roles in the regulation of adaptive responses to stress. These include brain stem noradrenergic neurons, sympathetic andrenomedullary circuits, and the parasympathetic systems [10-12]. However, in this thesis; we will focus only on HPA axis activation by assessing cortisol secretion levels in the aged population.

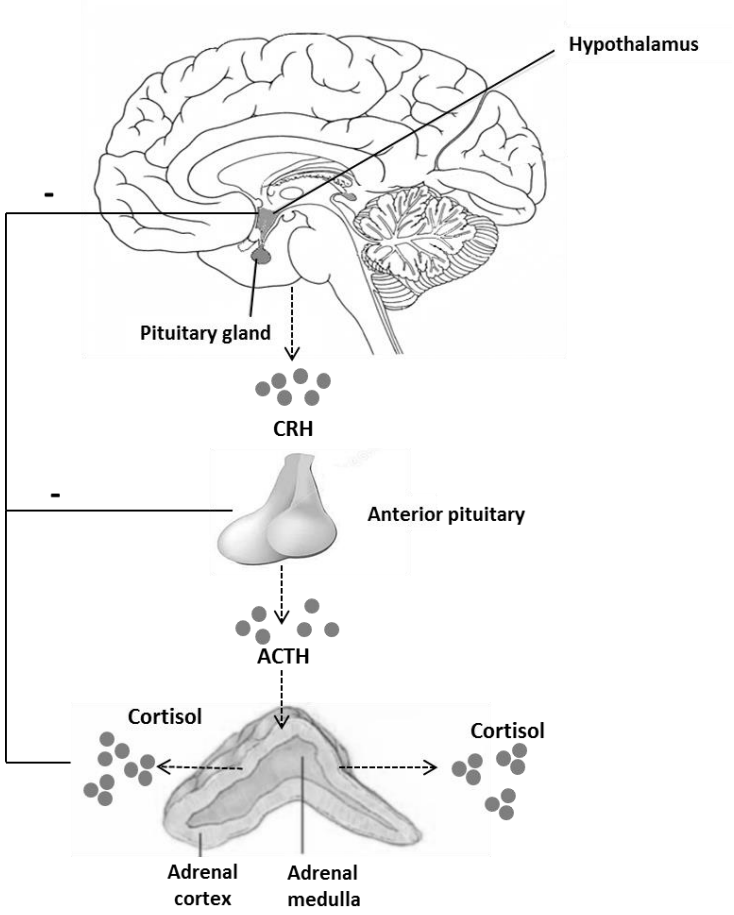

Figure 2 A simplified overview of neuroendocrine regulation of cortisol by the hypothalamic-pituitary adrenal (HPA) axis. The influence of stress and circadian rhythm trigger the neurosecretory cells in the PVN of the hypothalamus to release CRH which thereby stimulates the ACTH production from the pituitary. ACTH then induces the adrenocortical cells to secrete cortisol. Cortisol exerts its actions in maintaining various physiological homeostasis in the body. Circulating cortisol inhibits its own production both at the hypothalamic and the pituitary level as a negative feedback mechanism.

1.3 HPA axis regulation

The regulatory mechanism in cortisol secretion includes negative feedback mechanism and the regulation of circadian rhythm. Cortisol itself plays a vital role in regulating the HPA axis through a negative feedback loop which dampens the synthesis and release of CRH and ACTH. The negative cortisol feedback inhibition occurs at both pituitary and hypothalamus which include fast, delayed or slow feedback time domains [13]. The fast feedback action is within seconds to minutes for the rate of GC to increase in plasma following inhibition of stimulated ACTH and CRH release, not synthesis occurs when plasma GC levels are increasing [14]. For example, cortisol given to patients at the start of surgery attenuates the surgery-induces ACTH rise [15]. The delayed feedback occurs within 30 minutes to hours which affects corticotroph cells in the pituitary and hypothalamus and occurs after administration of one dose of GC [2, 16]. Unlike ACTH, CRH synthesis and release may be affected by this delayed feedback [15]. The slow feedback takes days or weeks and affects both basal and stimulated HPA axis activity and acts via glucocorticoid receptor (GR) mechanisms [13]. It is most important after long exposures to a moderately high dose of GC [15].

Circadian rhythmicity is a physiological hallmark of the dynamic feedback characterized by an endogenous oscillation within a near 24-hour period which is generated in the suprachiasmatic nucleus of the hypothalamus network [17, 18]. The circadian rhythm helps to adjust our body activities to the regular day and night periodicity. As depicted in **Figure 3**, a strong basal cortisol diurnal rhythm is typically characterized by high levels in the morning upon waking, which increase 50 - 60% in the first 30 - 45 min after awakening (the cortisol awakening response, or CAR). Levels steadily drop over the first few hours after waking, and then slowly decline throughout the day reaching nadir at midnight [19, 20].

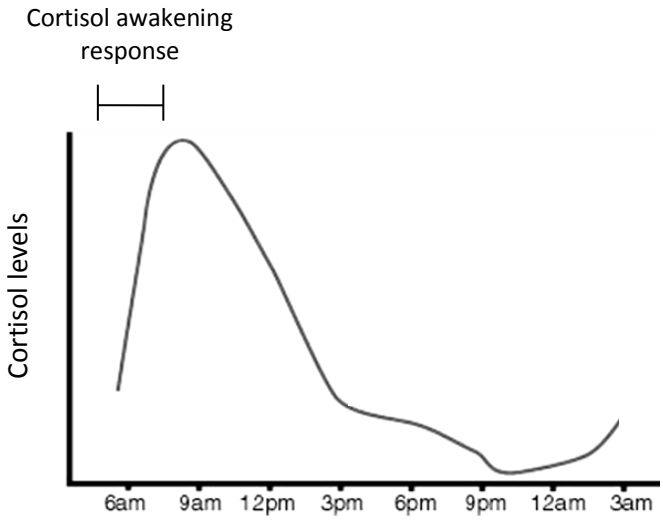

Figure 3. Normal cortisol diurnal rhythm

1.4 Cortisol Awakening Response (CAR)

The cortisol awakening response (CAR) refers to the sharp increase in cortisol release over the first 30 min after awakening [21]. The CAR can be measured as the difference between these two cortisol measures (specifically, the value of the 30 - 45 min after awakening sample minus the value of the wakeup sample). Another approach is to calculate the area under the curve (using the wakeup value as the baseline such that the CAR measure reflects an increase from the wakeup value) [22]. Research relating CAR and health showed a number of result discrepancies and the exact role of the CAR has still not been fully clarified. Low CAR has been associated with chronic health problems, posttraumatic stress and chronic fatigue syndrome while high CAR has been related to job stress and general life stress [23]. Adam et al. (2006) have suggested that it is an adaptive response aimed to provide the individual with the boost needed to meet the anticipated demands of the upcoming day [24]. Likewise, Fries et al. (2009) have suggested that the CAR may accompany an activation of prospective memory representations at awakening, enabling individuals to orient themselves in space and time, and helping them anticipate upcoming demands [21]. It has been suggested that the CAR may be regulated by different neurobiological mechanisms than the rest of the underlying diurnal cortisol curve [25].

1.5 Late evening cortisol

Late night saliva samples are generally collected by the patient at the time when the cortisol nadir is expected (2300 – 0000 h). An elevated late evening cortisol level has been linked to impairment in negative feedback of the HPA axis activation. Several investigators have shown that a high late night serum cortisol measurement has excellent diagnostic sensitivity (92 – 100%) and specificity (92 – 100%) in patients with suspected Cushing's syndrome [26, 27]. Late night salivary cortisol has been shown to have a superior diagnostic performance than urinary free cortisol measurement [28].

Besides the activation of the HPA axis that occurs in true pathologic Cushing's syndrome (see 1.7 below), psychiatric disorders (depression, anxiety disorders, and obsessive–compulsive disorder), poorly controlled diabetes mellitus and pregnancy are all associated with elevated late-night salivary cortisol levels [29-32]. However, increasing age and certain diseases, including type 2 diabetes mellitus, result in increased elevated cortisol levels and blunted cortisol diurnal variation, and it is not clear if current thresholds for abnormal late night cortisol results can be applied to such populations [32]. Data from KORA-Age population demonstrate an age-associated increase in late night salivary cortisol level in the normal, aged population [33]. Thus, this must be taken into account when applying traditional cut-off values during screening for Cushing's syndrome.

1.6 Measures of cortisol diurnal rhythm

Diurnal cortisol slope is best measured as the rate of decline in cortisol levels across the day, typically across the entire span of time from wakening to bedtime. Some researchers prefer to measure the diurnal cortisol pattern starting from the peak value of the day, taken 30 - 40 min after waking. However, no previous research has explored the ratio of peak morning to evening cortisol levels as a representation of the diurnal pattern. Which of these approaches are the most meaningful remains to be determined in further research. The minimal protocol for estimating a diurnal cortisol slope includes two data points–one in the morning (either wakeup or 30—40 min post-awakening) and one in the evening, with a slope calculated by subtracting bedtime from wakeup values, and dividing by the number of hours separating these two samples [34]. Resulting coefficients are negative, reflecting the declining slope of cortisol values [34]. The diurnal slope is the most commonly employed cortisol measurement with a steeper decline of diurnal slope typically associated with better psychosocial and physical health [34]. A flattened

diurnal slope has been shown in elderly subjects with poorer physical performance [35], lower cognitive performance [36, 37], type 2 diabetes [38], obesity [39] and who were socially isolated [40].

1.7 Age and sex differences in the HPA axis activation

In a meta-analysis of 45 studies, Otte et al. found an increased cortisol response to challenge in older

compared to young subjects [41]. A large body of evidence shows that an increased cortisol levels are associated with a variety of age-related diseases [41]. The mechanism that accounts for increased cortisol responses in the elderly is still unclear. However, according to the glucocorticoid cascade hypothesis [42], increased cortisol in the elderly is due to loss of mineralo- and glucocorticoid-receptors in the hippocampus, weakening the normal inhibitory role of the hippocampus on the HPA axis [43, 44]. The meta-analysis by Otte et al. also revealed a three-fold stronger effect of aging on cortisol responses to challenge in women compared with men [41]. However, Kudielka et al. provided evidence in a comprehensive review indicating that adult men respond to psychological stress with greater increases in cortisol compared to women [45]. Although the mechanism is not known, the effect of sex hormones may contribute to the increased cortisol response to challenge in women [41]. Thus, focusing on the HPA axis may offer novel therapeutic strategies to reduce morbidity in the elderly, albeit with adequate sex-specific targets.

1.8 Chronic Stress and Glucocorticoid (GC) Action

Chronic hypersecretion of cortisol may lead to symptoms known as Cushing's Syndrome (CS). Cushing's syndrome is relatively rare with earlier literature reports an incidence of 2-3 cases per million per year [46-48]. However, recent studies of high-risk groups report a significantly greater prevalence. Hypercortisolism has been reported in patients with hypertension, uncontrolled diabetes, adrenal masses, osteoporosis and vertebral fractures [49-53]. It is however still unclear whether the prevalence increase is due to higher-sensitivity testing, a higher recognition of disease in high-risk groups, or variability in the diagnostic criteria between previous and more-recent studies.

Chronic hypercortisolism may result in the accumulation of visceral fat consequently causing adiposity/obesity [54, 55]. GCs increase the activities of enzymes involved in fatty acid

synthesis and promote the secretion of lipoproteins [56, 57], induce the hepatic gluconeogenic pathway [58], promote the differentiation of preadipocytes to adipocytes, which could lead to an increased body fat mass [59], inhibit an insulin-stimulated amino acid uptake by adipocytes [60], and increase lipolysis or lipid oxidation which leads to the peripheral insulin resistance [61]. Thus, dysregulated cortisol secretion has been shown to be associated with reactive insulin hypersecretion, an increasing visceral obesity, and sarcopenia, resulting in dyslipidemia, hypertension, and T2DM [62]. Glucocorticoids also directly inhibit pituitary growth hormone, gonadotropin and thyrotropin secretion and make the target tissues of sex steroids and growth factors resistant to these hormones [63]. Thus, glucocorticoids antagonize the beneficial actions of GH and sex steroids on fat tissue (lipolysis) and muscle and bone anabolism [63].

On the opposite spectrum of Cushing's syndrome, clinically characterized hypocortisolism or primary adrenal insufficiency, also known as Addison's disease (AD) which can be caused by adrenal cortex dysfunction or secondarily a deficiency in pituitary ACTH secretion. The rare AD has estimates of prevalences in Caucasians from 39 to 117 per million [64]. Symptoms of AD are unspecific but mainly are fatigue, weight loss, hypotension and hypoglycemia and the main treatment of AD is with hydrocortisone administration [65, 66].

1.9 Salivary cortisol as a biomarker in stress research

Cortisol in saliva is in equilibrium with plasma free cortisol and unlike serum cortisol, is not affected in a major way by serum binding proteins. Salivary cortisol levels correlate well with the amount of free cortisol in the plasma at the time of collection. Saliva samples are generally collected at a specified time whereby subjects may chew a cotton swab which they then place in a collection tube. The tube is sealed and transported to the laboratory. Cortisol is rather stable in saliva and can be transported by standard postal services at ambient temperature [67]. Alternatively, the sampling device or saliva can be frozen for storage and later analysis. Several methods are available for the measurement of cortisol in saliva, including radioimmunoassay [68], enzyme-linked immunoassay [69], non-isotopic automated immunoassay [70], and liquid chromatography–tandem mass spectrometry [68].

The salivary cortisol assessment truthfully follows intra- and interindividual variations in plasma, which significantly contributes to its usefulness as a psychophysiological readout [71,

72]. This supports salivary cortisol assessment as a reliable measurement to be conducted in a population-based setting. Besides that, the integrity of the HPA axis can be evaluated using a variety of paradigms in basal and challenge conditions. As an indicator of basal HPA axis functioning, the saliva cortisol day curve, the late night measurement, and cortisol awakening response (CAR) were assessed in many studies [34]. In addition, neuroendocrine challenge tests have been developed to study HPA axis activation including the dexamethasone suppression test (DST), which examines whether ACTH production by the pituitary can be inhibited by the oral administration of dexamethasone [73].

Background to the specific topics of the thesis

1.10 Frailty: epidemiology and risk factors

Frailty is a multidimensional syndrome characterized by increased vulnerability to stressors as a result of cumulative age-related decline in many physiological systems occurring during the lifetime. Frail elderly have a substantially increased risk of adverse health outcome including falls, disability, long-term care, and mortality [74, 75]. The most commonly used definitions were based on physical performance of gait speed, grip strength, unintentional weight loss, and sedentary behaviour as well as mental health features of feeling of exhaustion and fatigue [74] although cognition [76] and nutrition [77] have been included by others too.

A substantial variation of frailty prevalence rates of 4.0-59.1% were reported in a recent systematic review (age ≥ 65 years) which largely explained by the difference in the operational definitions for frailty and the study variations [78]. The weighted average prevalence rate was 9·9% (95% CI 9·6–10·2) for frailty and 44·2% (44·2–44·7) for pre-frailty [78] in studies that used the phenotype model. Frailty was more prevalent in women than in men and steadily increasing with age: 65–69 years 4%; 70–74 years 7%; 75–79 years 9% 80–84 years 16%; older than 85 years 26% [78]. Most frailty models were developed in Caucasian populations, and the prevalence of frailty might be higher in people living in southern Europe [79] and in elderly Hispanic and African–American populations [80]; therefore, different cutoffs might be necessary for assessing frailty in different populations.

Neuroendocrine alterations are thought to be involved in the etiology of frailty, but have not been well characterized in previous studies which were limited to either sex-specific samples or

study size [81, 82]. Recent meta-analysis demonstrated associations between greater diurnal cortisol decline and gait speed [35]. A link between hypercortisolism and frailty is plausible due to the association of high cortisol levels and increased catabolism, leading to loss of muscle mass, anorexia, weight loss, and reduced energy expenditure, all of which are key clinical features of frailty [83]. Thus, frailty manifests as the failure in homeodynamic cycles around energy metabolism and neuromuscular changes with alteration in protein synthesis leading to sarcopenia. Increased rates of sarcopenia has also been shown in diabetes-related physical frailty [84].

1.11 Cognitive impairment: epidemiology and risk factors

Cognitive impairment is the decline of intellectual functions which covers various functional domains mainly orientation, memory, attention or calculation and language. The effects range from mild forms termed as mild cognitive impairment (MCI) that is not accompanied by any significant functional dis-ability and defines a transitional stage of between normal cognitive function and dementia [85]. Dementia is characterized by severe cognitive which is enough to compromise social and/or occupational functioning [85].

Prevalence of dementia increases exponentially with increasing age, and doubles every five years of age after age 65 [85]. In higher income countries, prevalence is 5–10% in those aged 65+ years and generally greater among women than among men, due to longer life expectancy of women than men [85]. Risk factors including lower education, hypertension, high cholesterol, high body mass index (BMI) and disease conditions of cardiovascular diseases, coronary heart diseases, stroke and obstructive sleep apnea are associated with increased risk of dementia in late life [85]. Psychological risk factors which are associated with increased risk of dementia are depression, anxiety, post-traumatic stress disorder. The Apolipoprotein E (APOE) gene polymorphism on Chromosome 19 has been identified as increasing susceptibility for dementia as well as Alzheimer's disease [86, 87].

Dysregulation of the HPA axis by psychological stress, which results in chronically high cortisol exposure of the hippocampus, is proposed to contribute to cognitive decline in the elderly [88]. An elevated circulating level of cortisol has been implicated in the pathogenesis of cognitive impairment through detrimental effects of glucocorticoids on hippocampal neurons [88, 89]. Apart from the damage in the hippocampal and prefrontal region through GC receptor-

mediated effects, altered glucose transport into the brain has a key role in the underlying mechanism of decline in cognitive function [90]. Thus, there is a link between HPA axis dysfunction, cognitive impairment and metabolic dysregulation leading to type 2 diabetes.

1.12 Sleep and cognitive decline

Sleep is a complex behavioral state that occupies one-third of the human lifespan. Many studies have identified the protective factors influencing sleep that could be grouped under three broad categories, namely socioeconomic, behavioral, and nutritional [91]. There is growing evidence on a potential role of good sleep quality as one of the modifiable risk factor of cognitive decline [92]. Sleep was thought to be important primarily for restoring brain function. However, increasing evidence suggests that sleep also modulates the metabolic, endocrine, immune and cardiovascular systems. Sleep is regulated by a homeostatic factor and by a circadian factor, which facilitates falling asleep in the evening [93]. Interactions between sleep and the circadian clock regulate hormonal control including the HPA axis with cortisol [94]. In a systematic review, prospective studies have identified female gender, depressed mood, and physical illness as general risk factors for future sleep disturbances in later life [95]. Alterations in hormonal profiles in aging process are linked to the development of cognitive impairment, through the inability of the body to return to a basal condition of homeostasis after stressful conditions [96]. Therefore, sleep quality plays a role on the neuroendocrine activity which allows for the optimal regulation of the hormonal homeostasis [97].

2. Rationale and methods

2.1 Specific aims

The thesis is based on the hypothesis that dysregulated cortisol secretion, represented by specific cortisol levels taken at various times during the day, is related to the frailty phenotype and cognitive impairment in the elderly. We also explored the role of impaired sleep patterns in predicting cognitive decline in a prospective analysis with a 3-year follow up in the aged population.

The specific objectives were:

Manuscript 1: to examine the association between salivary cortisol secretion and frailty phenotype in a representative elderly population of KORA-Age.

Manuscript 2: to assess the association between salivary cortisol and cognitive function in a representative elderly population of KORA-Age.

Manuscript 3: to investigate whether major patterns of poor sleep (difficulties in initiating sleep (DIS), difficulties in maintaining sleep (DMS) or both DIS and DMS, daytime sleepiness, and sleep duration) were associated with cognitive decline in a population-based sample of older adults over 3 years of follow up.

2.2 Study population

The data in this cumulative thesis stem from the KORA (Cooperative Health Research in the Region of Augsburg)-Age study which was a follow-up study of the four MONICA/KORA Augsburg Surveys, conducted between November 2008 and November 2009 [98]. All participants, aged 64 years or older, were selected from a population-based random sample (N = 5991) of the previous surveys (Survey 1-4, conducted between 1984 and 2001 in the Augsburg region, Southern Germany) as displayed in **Figure 4**, with participation rates ranging between 67% and 79% [98]. In total, 4127 people participated in a standardized telephone interview. Out of this group, a randomly drawn sample of 1079 participants additionally underwent extensive physical examinations including collection of blood samples, anthropometric examination, and a personal interview. In 2012, a re-examination was performed in 822 persons (84.3% of all

eligible persons). Both baseline and follow-up surveys included a telephone and personal interview and a physical examination which assessed somatic and mental health related multimorbidity.

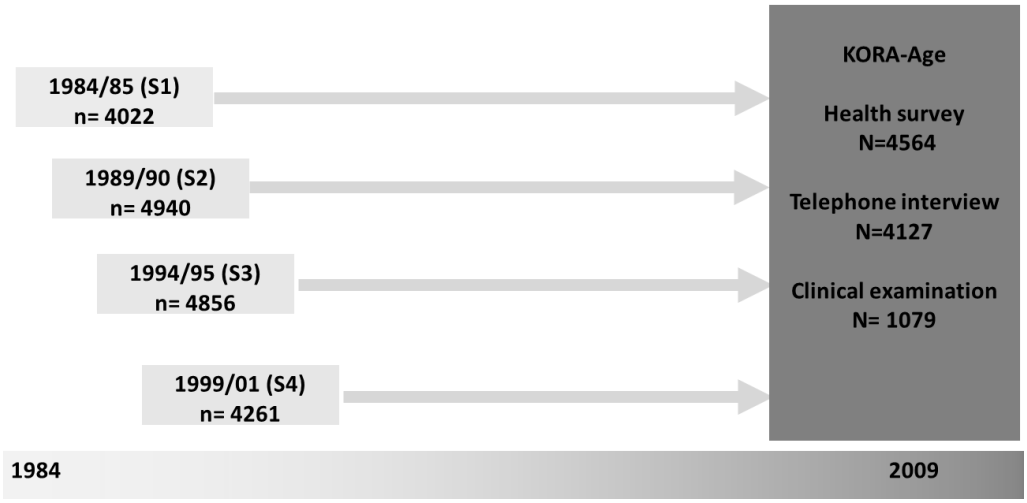

Figure 4. KORA-Age study and the previous MONICA/KORA surveys (1-4)

For the analysis of manuscript 1 and 2, only participants who provided complete, salivary samples (N = 772, saliva sampling rate of 72%) were included. The final data set for manuscript 1 consisted of 722 participants (382 males and 363 females) aged 65 to 89 years (mean age =75 years) and manuscript 2 consisted 733 participants (375 males and 358 females) aged 65 to 89 years (mean age =75 years). For manuscript 3, a total of 740 study participants (men=49 %, N= 366, women 51%, N=374, mean age=75 years (range 64-94 years) with complete data on baseline covariates and cognitive status as well as cognitive status at follow up were included in the study. The KORA-Age study was approved by the Ethics Committee of the Bavarian Medical Association and all participants provided a written informed consent.

2.3 Statistical analyses

Manuscript 1 uses multiple linear regression models to assess the association of frailty counts and cortisol levels (morning after awakening (M1), 30 minutes after awakening (M2), late evening (E), ratio of M1 to E (M1/E), and ratio of M2 to E (M2/E)). All covariates and cortisol measures were entered into a multinomial logistic regression model with the outcome frailty to distinguish the risk of being frail or pre-frail (as opposed to non-frail) and then we further employed a logistic linear regression with the outcome of each specific frailty criteria (weight loss, exhaustion, physical inactivity, walking speed and Timed Up and Go test).

In **manuscript 2**, cortisol measures and important covariates, with the outcome cognitive (TICS-m) score were entered into a multiple linear regression model to assess the effect of cortisol levels on the risk of increased cognitive score. A sex-stratified multiple variable linear regression analysis was also considered. An additional multinomial logistic regression analysis was computed with a categorical cognitive status variable as the outcome, to distinguish the risk of being mildly cognitively impaired or probably demented (as opposed to having normal cognitive function).

In **manuscript 3**, we employed multiple linear regressions to analyze the association between various patterns of poor sleep (main exposure) and cognitive change (continuous outcome) after 3 years of follow up. Three distinct patterns of poor sleep (sleep difficulties at night (DIS, DMS and both), daytime sleepiness, and sleep duration) and the total sum score of all sleep domains were considered in separate regression models. We then examined the interaction of the effect of any of the three patterns of poor sleep with baseline cognitive status on cognitive decline, by including the product of both variables in a fully adjusted model (adjusted for age, sex, baseline cognitive score, smoking status, alcohol consumption, physical activity, depressive symptoms, hypertension, and somatic co-morbidities).

3. Results

3.1 Manuscript 1: Blunted diurnal cortisol pattern is associated with frailty: a cross-sectional study of 745 participants aged 65 to 90 years *(H. Johar et al., Journal of Clinical Endocrinology and Metabolism, 2014)*

In manuscript 1, lower cortisol levels in the morning after awakening sample (M1) (P =0.18) and 30 minutes after awakening, M2 (P = 0.14) and increased evening cortisol levels (P = 0.004) were observed in pre-frail (35.17%, n=262) and frail (3.36%, n=25) individuals, in a dose-response manner. Frailty was strongly associated with smaller ratios of morning to evening levels; M1 to E ratio (P=0.02) and M2 to E ratio (P=0.003). Higher evening cortisol levels were associated with a 24% increased risk of a pre-frail state (odds ratio, 1.22; 95% confidence interval, 1.03–1.44). In fully adjusted multinomial logistic regression models calculated for each of the 5 individual frailty criteria as outcome, an increased risk of low gait speed was associated with increasing evening cortisol levels (1.35, 1.10–1.65, per 1-standard deviation (SD) increase, P = 0.004) as well as the ratio M2/E (1.42, 1.09–1.86, P = 0.01). A 51% increased risk of low physical activity was associated with an increase in CAR (1.51, 1.14–2.01, P = 0.004), and risk of low grip strength was associated with both ratios of M1/E (1.36, 1.03–1.79, P = 0.03) and M2/E (1.31, 1.02–1.68, P = 0.04).

3.2 Manuscript 2: Lower morning to evening cortisol ratio is associated with cognitive impairment in men but not women: An analysis of 733 older subjects of the cross-sectional KORA-Age study. *(H. Johar et al., Psychoneuroendocrinology, 2015)*

In manuscript 2, a dose response finding was observed with lower morning (M1 and M2), and increased evening levels in participants with probable dementia (4.5%, N = 33) and slightly increased in mildly cognitive impaired (MCI) (13.8%, N = 101) compared to healthy individuals. Higher morning to evening ratios were associated with reduced odds of cognitive impairment, even after adjustments for important confounders (M1/E ratio: OR = 1.50, 95% CI = 1.08—2.07, M2/E ratio: 1.41, 1.01—1.95, per 1-SD increase). In a sex-stratified analysis, M1, M2, E levels and both ratios of M1/E and M2/E levels were significantly associated with cognition in men and not women. In fully adjusted model, higher M1/E and M2/E ratios were

significantly associated with increased odds of better cognition (M1/E: OR = 1.94, 95%CI = 1.24—3.02, P= 0.004; M2/E = 1.74, 1.12—2.71, 0.01) in men but not women (M1/E = 1.11, 0.69—1.78, 0.67; M2/E = 1.09, 0.67—1.77, 0.74).

3.3 Manuscript 3: Impaired Sleep Predicts Cognitive Decline in Old People: Findings from the Prospective KORA Age Study. *(H. Johar et al., SLEEP, 2015)*

Using a prospective analysis approach, manuscript 3 investigated the predictive role of poor sleep patterns with cognitive decline. In this investigation, participants who reported problems maintaining sleep (n=211) were more likely to have a decline in their cognitive score after 3 years compared to those who reported no DMS at baseline (fully adjusted model, β=0.88, 95%CI=0.03-1.74, P = 0.04). Given the significant interaction between cognitive decline and sleep disturbances (DMS*baseline cognitive status, P=0.04), we further stratified our analyses according to normal or impaired cognitive status at baseline. DMS was significantly associated with cognitive decline only in participants with normal cognitive status and not in cognitively impaired individuals. In a fully adjusted model, cognitive decline was 1.3-points larger in cognitively normal individuals with DMS compared to individuals with no sleep disturbances (β=1.26, 95%CI=0.35-2.18, P=0.007) whereas the effect in cognitively impaired participants was not statistically significant in the model (β=0.06, 95%CI=-2.23-2.35, P=0.96).

4. Conclusion and outlook

Psychological stress, by the activation of the HPA axis, plays a key role in the development of frailty and cognitive impairment. This thesis presented novel findings on the detrimental effects of dysregulated cortisol, in specific cortisol secretion patterns, on two important age-related conditions, namely the frailty syndrome and cognitive function as presented in manuscripts 1 and 2, respectively. Firstly, this thesis confirms and expands the view that blunted diurnal cortisol responses were observed in both men and women with increasing frailty burden. Findings from manuscript 1 successfully identify that the lack of diurnal variability, as reflected by a lower morning to evening cortisol ratio, is associated with frailty burden. Our observations support the predominant role of muscle atrophy, reflected in the grip strength and gait speed criteria, and cortisol dysregulation even in the pre-frail state.

Secondly, manuscript 2 similarly highlights the role of reduced diurnal variability with lower morning and higher evening cortisol levels in the pathophysiological mechanism of cognitive impairment in the elderly. However, the significant association of an increased risk for cognitive impairment was observed among men but not women. A blunted diurnal cortisol pattern appears to be a sensitive measure of decreased HPA axis resilience that leads to diminished cognition that can be observed even in MCI individuals. In the same vein of manuscript 1, the simple morning to evening cortisol level ratios which is relatively unexplored, were sensitive to detect an association of impaired HPA axis functioning and cognition. Thus, it is important to assess the clinical relevance of the morning and evening cortisol balance as a therapeutic target. Since the nature of cross-sectional studies of both manuscript 1 and 2 cannot infer causality, a prospective analysis of the study population is warranted.

Cortisol is essentially involved in many physiological processes mainly aimed at energy mobilization. Energy metabolism and increases in proinflammatory cytokines are potential biological mechanisms which are shared by both frailty and cognitive dysfunction. Alterations in protein synthesis and protein breakdown in muscles lead to an increased rate of muscle mass loss is related to diabetes [84]. Damage in the hippocampal and prefrontal area through GC receptor-mediated effects and by affecting glucose transport into the brain are also linked to type 2 diabetes [90]. Diabetes is a relevant midlife epidemic that

predisposes individuals to frailty and cognitive decline [4, 67] . Thus, we further investigate the link between cortisol secretion patterns, type 2 diabetes and glycemic control in the KORA-Age population and found an elevated late evening cortisol patterns in subjects with type 2 diabetes as well as sex-specific association of enhanced Cortisol Awakening Response (CAR) in men [99]. We also found that the association of higher CAR in men was influenced by central adiposity [99]. Overall, the impact of dysregulated cortisol secretion on frailty and cognition is likely a consequence of a multitude of interrelated factors, of which only some have been evaluated within the scope of this dissertation. In addition, longitudinal data of the 3 year follow up of KORA-Age 2 will provide further insight into the order of events with respect to HPA axis abnormalities.

As a result of the important link between HPA axis and cognitive function, findings from the prospective analysis in manuscript 3 confirm and extend the existing literature on the role of sleep as a modifiable risk factor for cognitive decline. Sleep is important primarily for restoring brain function as well as modulating metabolic, endocrine and cardiovascular system. Furthermore, sleep deprivation is a clear example of a stressor which has detrimental effects on HPA activity [96]. Given the close relationship between frailty and cognitive function [100], sleep has been also suggested recently to play a role in muscle protein metabolism [101]. Reductions in sleep quality and duration as well as increases in prevalence of circadian-related sleep disorders with age favor proteolysis, altered body composition, and increase the risk of insulin resistance, all of which have been associated with sarcopenia and frailty.

In conclusion, this thesis has outlined the underlying psychoneuroendocrinology mechanism leading to frailty and cognitive impairment as well as sleep deprivation as a potential contributor leading to cognitive decline in the elderly. Furthermore, understanding the link between frailty and cognitive function has implications for the management of elderly patients. Alterations in cortisol secretion patterns that occur during the aging process are implicated in the development of frailty and cognitive impairment through a variety of mechanisms involving the adaption to stressful conditions. Stressful experiences increase levels of stress hormones in older individuals who may be less resilient, and unable to return to basal conditions of homeostasis. Moreover, non-pharmacological stress management and

lifestyle interventions have shown beneficial to improve the well-being of pre-frail and MCI individuals, as well as to improve sleep quality and overall health in the elderly [102-104].

The novel morning to evening cortisol ratio as a marker of HPA axis activity may provide crucial information about the underlying mechanism of frailty and cognitive impairment. The ability to identify pre-frail and MCI individuals requires more attention as these elderly people, who are most at risk for worse prognoses, are a critical target for intervention strategies. Moreover, the identification of impaired sleep quality is critically important for optimal maintenance of health in older age. Finally, future research should focus more on the relevant role of chronic exposure of psychological stress leading to the activation of HPA axis in order to better understand the underlying biological mechanism of adverse age-related conditions. Our current understanding on the association between chronic stress, HPA axis dysregulation and metabolic dysregulation which includes type 2 diabetes and obesity, both linked by inflammation, opens new research avenues that we hope to proceed in near future. Furthermore, we plan to incorporate dynamic and multivariate methods for assessing links between stressors and other potential neuroendocrine biomarkers of dehydroepiandrosterone sulfate (DHEAs), insulin-like growth factor (IGF) and melatonin in our future work endeavors.

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6. Paper 1: Blunted Diurnal Cortisol Pattern and Frailty

Original title: Blunted Diurnal Cortisol Pattern Is Associated With Frailty: A Cross-sectional Study of 745 Participants Aged 65 to 90 Years

Authors: Hamimatunnisa Johar, Rebecca T. Emeny, Martin Bidlingmaier, Martin Reincke, Barbara Thorand, Annette Peters, Margit Heier, and Karl-Heinz Ladwig

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Brief Report—Endocrine Research

Blunted Diurnal Cortisol Pattern Is Associated With Frailty: A Cross-sectional Study of 745 Participants Aged 65 to 90 Years

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Background: The role of neuroendocrine alterations in the etiology of frailty syndrome is still poorly understood. Hypothalamic-pituitary-adrenal axis dysregulation is a plausible candidate pathway contributing to frailty. Thus, we sought to examine the associations of diurnal cortisol secretion with frailty in older adults.

Methods: A cross-sectional analysis was conducted among 745 study participants (age 65–90 years, mean age 75.1 years) of the population-based KORA Age study. Associations between salivary cortisol measures at awakening (morning 1 [M1]), 30 minutes after awakening (M2), and evening (E) and frailty criteria were determined.

Results: Lower cortisol levels in the first morning sample (M1) ($P = 0.18$) and M2 ($P = 0.14$) and increased E levels ($P = 0.004$) were observed in prefrail (35.17%, n = 262) and frail (3.36%, n = 25) individuals, in a dose-response manner. Frailty was strongly associated with smaller ratios of morning to evening levels; M1 to E ratio ($P = 0.02$) and M2 to E ratio ($P = 0.003$). Higher evening cortisol levels were associated with a 24% increased risk of a prefrail state (odds ratio, 1.22; 95% confidence interval, 1.03–1.44). A smaller morning to evening ratio was associated with an increased risk of low grip strength (1.42, 1.09–1.86) and gait speed (1.31, 1.02–1.68).

Conclusion: Frailty status is associated with blunted cortisol reactivity as demonstrated by lower morning and higher evening salivary cortisol levels.

Frailty is a geriatric paradigm that is not disease-specific but represents systemic dysregulation and decline in functional health. It is characterized by unintentional weight loss, feeling of exhaustion and fatigue, physical inactivity, slow gait speed, and low grip strength (1). Frailty confers a high risk for adverse outcomes such as functional dependency, institutionalization, and increased risk of mortality (1). To date, little is known about the mechanisms leading to frailty. In a recent meta-analysis (2), a greater diurnal decline of the hypothalamic-pituitary-adrenal (HPA) axis was associated with better physical performance in later life. This was also reflected by the Whitehall II cohort, in which a flatter diurnal pattern was associated with poorer health outcomes in older adults (3). Thus, impaired neuroendocrine regulation with increased vulnerability to stressors is a potential contributor to physiologic dysregulation observed in frail states (4). Furthermore, altered diurnal cortisol pattern indicated by lower morning and high cortisol in the late evening (5) may contribute to the development of sarcopenia, which likely contributes to frailty (6).

Neuroendocrine alterations are thought to be involved

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Abbreviations: BMI, body mass index; CAR, cortisol awakening response; DCS, diurnal cortisol secretion; E, evening; HPA, hypothalamic-pituitary-adrenal; morning 1, M1.

in the etiology of frailty but have not been well-characterized in a large community-dwelling population. Therefore, we sought to examine the dynamics of diurnal cortisol secretion (DCS) in frail, prefrail, and healthy study participants. Variations in characteristics of the study population such as multimorbidity (7), age, sex, smoking, body mass index (BMI) (8), and medications (9) affect cortisol levels and thus were considered as confounders in the association investigated between frailty and DCS. In particular, we tested the following hypotheses: (i) frailty criteria are associated with DCS and (ii) less variance of DCS is associated with frailty due to impaired neuroendocrine regulation.

Materials and Methods

Study setting and population

The KORA Age study was conducted between October 2008 and February 2009 and was approved by the Ethics Committee of the Bavarian Medical Association. All participants, aged more than 65 years, were selected from the previous surveys (Survey 1–4, conducted between 1984 and 2001) with participation rates ranging from 67% to 79% (10). For the current analysis, a standardized interview and medical examination were administered to 1079 study participants by trained medical staff, and only participants who provided complete, plausible salivary samples were included (n = 722, saliva sampling rate of 27%; see Supplemental Table 1, published on The Endocrine Society's Journals Online website at http://jcem.endojournals.org). Thus, 382 males and 363 females (age 65–90, mean age 75.1 years) were included in the analysis. In a dropout analysis of the excluded participants, no significant age and sex differences were observed (data not shown).

Salivary cortisol

Participants were individually instructed about the saliva sampling procedure and provided with additional detailed written information (Salivette salivary sampling test kit). Three saliva samples were assessed; in the morning after awakening (morning 1 [M1]), 30 minutes after awakening (M2), and in the late evening before bedtime (E). Cortisol levels were determined in duplicate using the Luminescence Immunoassay RE62011 (range 0.1–40 ng/mL, IBL) and the Victor Multilabel Plate Reader (PerkinElmer).

Frailty assessment

Participants were classified as frail if 3 or more of the following criteria proposed by Fried et al (1) were met, prefrail if 1 or 2 criteria were fulfilled, and nonfrail if none of the criteria applied: weight loss, loss of more than 5 kg in the past 6 months; exhaustion, a lack of feeling energetic and active over the last 2 weeks; physical inactivity, not performing any sports during summer and winter and walking less than 30 minutes daily; low walking speed, consuming the most time in the Timed Up and Go-Test (11) (in the highest quintile stratified according to sex and mean standing height); and weakness, lowest quintile (stratified according to sex and BMI) of the mean value of 3 grip strength measurements as determined using the JAMAR handheld dynamometer (Saehan Corp).

Covariates

Sociodemographic variables included age, sex and education. Low education was defined as less than 8 years of education. BMI was recorded as body weight in kilograms divided by the square meters of height. Someone who smoked cigarettes regularly or irregularly was considered as a current smoker. Multimorbidity was defined as the co-occurrence of more than two disease conditions on the Charlson Comorbidity Index (12).

Statistical analysis

Baseline descriptive analyses of demographic and clinical characteristics were stratified by frailty status. In case of nonnormality, tests were performed on log-transformed variables and results are presented as geometric means with antilog of SEs of the adjusted log means. Cortisol awakening response (CAR = M2 − M1) and the ratios of M1 to E (M1/E) and M2 to E (M2/E) were calculated. Least-squares means of cortisol measurements were calculated in age- and sex-adjusted models. Adjusted means with 95% CI are presented, and differences between groups were tested with general linear model procedures.

Multivariate linear regression models were used to assess the association of frailty and DCS (M1, M2, E, M1/E, and M2/E). All covariates and cortisol measures were entered into a multinomial logistic regression model with the outcome frailty to distinguish the risk of being frail or prefrail (as opposed to nonfrail) and then entered into a logistic linear regression with the outcome of each specific frailty criteria. Estimates from multinomial logistic regression analyses are expressed as increased risk per 1 SD of the respective cortisol measures.

Analyses were performed using SAS statistical software version 9.2 (SAS Institute) and P values $< .05$ were considered statistically significant. The STROBE (strengthening the reporting of observational studies in epidemiology) checklist was applied in preparation of the manuscript.

Results

Smaller M1/E ($P = 0.02$) and M2/E ($P = 0.003$) ratios were associated with frailty status in this present study. The commonly used CAR was not significantly different between the frailty categories. Compared with healthy individuals, frail (n = 25, 3.4%) and prefrail (262, 35.2%) individuals had lower mean of M1 ($P = .18$) and M2 ($P = .14$) and higher E ($P = 0.003$) cortisol levels (Table 1).

The associations of cortisol means (least-squares means adjusted by age and sex) with the 5 frailty criteria revealed that increased evening levels ($P = .002$) were observed in participants with slow gait speed, whereas lower morning levels ($P = .035$) were found in participants with low grip strength (Figure 1). In fully adjusted multinomial logistic regression models calculated for each of the 5 individual frailty criteria as outcome, an increased risk of low gait speed was associated with increasing evening cortisol level (1.35, 1.10–1.65, per 1-SD increase, $P = .004$) as well as

Table 1. Geometric Mean Concentration (Antilog of SE) of Various Cortisol Measures by Frailty States (n = 745)

Cortisol Measurement	Cortisol Concentration, ng/mL			
	Frail (25, 3.36%)	Prefrail (262, 35.17%)	Nonfrail (458, 61.48%)	*P* Value for Differences Between Groups[a]
M1	3.5 (2.4)	3.7 (1.8)	3.9 (1.8)	0.18
M2	4.2 (1.7)	4.8 (1.8)	5.2 (1.9)	0.14
E	0.9 (1.8)	0.8 (2.1)	0.7 (1.9)	0.004
CAR	1.2 (3.2)	1.17 (2.7)	1.4 (3.1)	0.17
M1/E ratio	5.2 (3.6)	6.72 (7.9)	7.9 (6.5)	0.02
M2/E ratio	6.6 (5.6)	8.52 (7.1)	10.5 (8.3)	0.003

[a] Adjusted for age and sex.

the ratio M2/E (1.42, 1.09–1.86, $P = .01$). A 51% increased risk of low physical activity was associated with an increase in CAR (1.51, 1.14–2.01, $P = .004$), and risk of low grip strength was associated with both ratios of M1/E (1.36, 1.03–1.79, $P = .03$) and M2/E (1.31, 1.02–1.68, $P = .04$). However, the association with both ratios was no longer significant if Bonferroni correction for multiple testing was applied (0.05/6, $P < .008$).

When the frailty construct was modeled in fully adjusted analyses, only increases in evening cortisol were associated with a 22% increased risk of prefrail vs healthy status (odds ratio, 1.22; 95% confidence interval, 1.03–1.44; $P = .02$). For the risk of being frail vs nonfrail, similar effects were observed, but results failed to show significance possibly due to the small number of frail participants (1.24, 0.83–1.83, $P = .3$). A sensitivity analysis with additional adjustment for chronic obstructive pulmonary disease, rheumatoid arthritis, and gastrointestinal disorders, which are associated with the use of glucocorticoid medications, did not alter the observed results (data not shown).

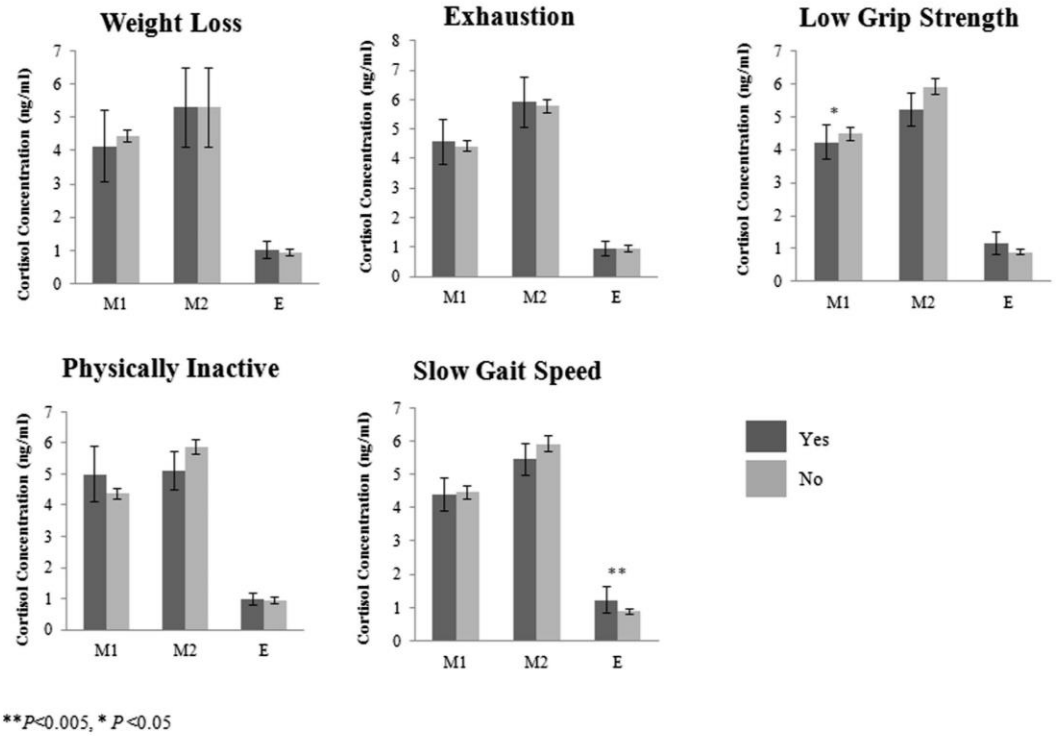

Figure 1. Adjusted geometric means of cortisol by frailty criteria.

Discussion

This study is the first to show evidence of a dysregulated DCS that features lower morning and higher evening cortisol levels in frail and prefrail elderly men and women from a large, community-based epidemiologic sample. This confirms the hypothesis that blunted cortisol levels (low reactivity) are associated with negative health outcomes (13). To the best of our knowledge, to date, only 2 studies have investigated this issue, among women (4) and in institutionalized elderly (14). In the first study, a smaller diurnal decline and higher evening cortisol were seen in women with greater frailty burden, whereas the second study reported higher cortisol values in both morning and evening samples from frail institutionalized elderly. Thus, our findings not only corroborate the results from the study among women but also expand the view that blunted diurnal cortisol responses were observed in both men and women with increasing frailty burden. Furthermore, the significance of the novel morning to evening cortisol ratios demonstrated in our study were likely influenced by the evening levels. This was recently demonstrated by a prospective study by Gardner et al (15), in which a higher evening cortisol level, which in turn contributed to less diurnal variability, predicted poorer physical performance in participants of the Caerphilly Study.

The dynamic regulation of DCS, indicating an individual's ability to adjust from the highest cortisol level in the morning to the lowest basal level at night, is clearly captured in the novel ratio concept introduced in this analysis. This ratio concept sharpens the current knowledge on the characteristics of DCS with flat or steep diurnal cortisol slope, CAR, and single morning or evening cortisol measurements. A smaller ratio (low morning and high evening levels) indicates a disrupted diurnal rhythm and is also comparable to a flatter diurnal pattern, which was observed in the older individuals of the Whitehall II cohort (3). The combination of morning to evening levels as a ratio appears to provide a better insight of diurnal cortisol rhythmicity and thus HPA axis reactivity. This supports the current hypothesis that a more dynamic HPA axis, rather than absolute cortisol levels per se, is more important in determining frailty status (2).

Among the 5 frailty criteria, grip strength and gait speed were significantly associated with altered morning to evening cortisol ratio but not weight loss, physical inactivity, and exhaustion. These results suggest a link of disrupted cortisol regulation and sarcopenia as the underlying pathophysiology of frailty (6). Our observations support the predominant role of muscle atrophy, reflected in the grip strength and gait speed criteria, and cortisol dysregulation in prefrail states. These results complement a recent meta-analysis that demonstrated associations between greater diurnal decline and gait speed (2). The clinical relevance of these findings is paramount because exercise and protein intake, which preserve and increase muscle mass, are successful first-line interventions for prefrail states (6). Furthermore, assessing frailty status in clinical settings may help in decision making for suitable patient-specific treatment (ie, invasive, conservative, or palliative care) because current disease-specific prognostic scoring systems are unable to differentiate between frail and nonfrail elderly (16). In a clinical setting, an assessment of frailty may be considered as time-consuming, and therefore, measurements of cortisol may offer a feasible alternative.

Study strengths and limitations

The strength of our study rests in the salivary sample collection from a large, sex-equivalent, representative population with a high study response rate. A disadvantage of this study is the low percentage (3.4%, n = 25) of frail elderly that were assessed. However, a wide range of prevalences have been reported in the literature (8%–59.1%) with lower prevalence observed using a physical frailty construct compared with a broader definition of frailty (2). We therefore cannot rule out a selection bias in this study, because only participants who were healthy enough could be assessed for gait speed. The easy and noninvasive salivary cortisol sampling method captures the DCS. Although the strictest quality control measures were taken, salivary sampling collection time deviance could be a potential source of variance adherent in the CAR measurement (17). Because the nature of cross-sectional studies cannot infer causality, a prospective analysis of the study population is warranted.

Conclusion

A blunted cortisol response of lower morning and higher evening cortisol levels reflects the altered HPA axis reactivity that contributes to the underlying pathophysiologic mechanisms leading to frailty. The novel morning to evening cortisol ratio identifies muscle atrophy as a prominent marker of prefrail individuals, a vulnerable patient population that would likely benefit from targeted interventions.

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K.-H.L. and M.B. designed the study and proofread the manuscript. M.R., B.T., A.P., and M.H. proofread the manuscript. R.E. advised on statistical analysis and proofread the manuscript. H.J. managed the literature searches and statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Disclosure Summary: The authors have nothing to disclose.

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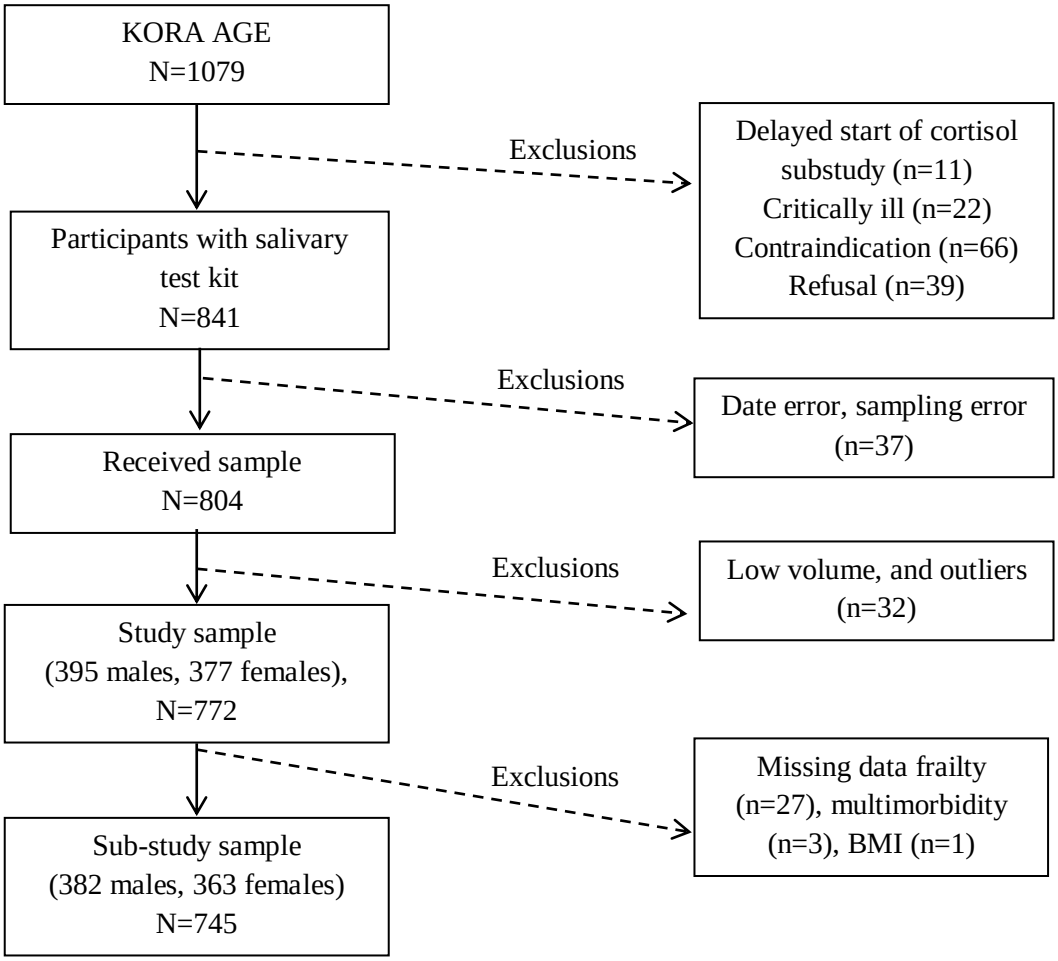

Supplementary Figure 1: Flow chart of the cross-sectional study design derived from the KORA (Cooperative Health Research in the Augsburg Region, Germany) Age-1 Study (2008-2009)

7. Paper 2: Lower Morning to Evening Cortisol Ratio and Cognitive Impairment

Original title: Lower morning to evening cortisol ratio is associated with cognitive impairment in men but not women: An analysis of 733 older subjects of the cross-sectional KORA-Age study

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Lower morning to evening cortisol ratio is associated with cognitive impairment in men but not women: An analysis of 733 older subjects of the cross-sectional KORA-Age study

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KEYWORDS
Cortisol;
Cognitive function;
Aging

Summary
Background: A dysregulated hypothalamic—pituitary—adrenocortical axis (HPA) is thought to play a role in the pathophysiology of cognitive impairment. Surprisingly, little agreement exists on the association of cortisol and cognitive impairment. Thus, we sought to examine the association between cognitive function and salivary cortisol levels in a representative sample of older men and women.
Methods: A cross-sectional analysis was conducted among 733 study participants (65—90 years old, mean age = 74.9) of the population-based KORA (Cooperative Health Research in the Region of Augsburg)-Age study. Associations were examined between cognitive function (determined by telephone interview for cognitive status-modified, TICS-m) and salivary cortisol measured upon waking (M1), 30 min after awakening (M2), and in the late evening (E).
Results: In a dose response manner, lower morning (M1 and M2), and increased evening levels were observed in participants with probable dementia (4.5%, $N = 33$) and slightly increased in

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those with mild cognitive impairment (MCI) (13.8%, $N = 101$) compared to healthy individuals. Higher morning to evening ratios were associated with reduced odds of cognitive impairment, even after adjustments for important confounders (M1/E ratio: OR = 1.50, 95% CI = 1.08–2.07, M2/E ratio: 1.41, 1.01–1.95, per 1 standard deviation (SD) increase). However, the significant association of an increased risk for cognitive impairment was observed among men (M1/E: OR = 1.94, 95% CI = 1.24–3.02; M2/E = 1.74, 1.12–2.71) but not women (M1/E: OR = 1.11, 0.69–1.78; M2/E = 1.09, 0.67–1.77).

Conclusion: Our findings suggest that dysregulated HPA axis reactivity, evidenced by blunted diurnal cortisol responses, are associated with impaired cognitive function in an aged population.

1. Introduction

The maintenance of good cognitive function is important for successful aging in older individuals. Dysregulated hypothalamic–pituitary–adrenal (HPA) axis and, in particular, impaired diurnal cortisol secretion is hypothesized as one of the underlying mechanisms for cognitive decline among the elderly (Lupien et al., 2007). Chronic exposure to glucocorticoid (cortisol in human) is neurotoxic and with advancing age, hypercortisolemic-induced neuroendocrine changes may contribute to deleterious effects on the hippocampus, which can affect memory performance and lead to cognitive decline (Lupien et al., 1998). Damage to the hippocampus may also lead to reduced inhibition of the HPA axis and thus to further hypercortisolemia (Kudielka et al., 2004). The prefrontal cortex is noticeably vulnerable to chronic exposure to glucocorticoid which may alter executive function abilities (for review, see Lupien et al., 2007), thus, it is important to consider other cognitive domains that may be affected by dysregulated cortisol. Various features of diurnal cortisol profiles have been applied to study adverse health outcomes, including the cortisol awakening response (CAR) and the diurnal cortisol slope. In general, a steep rise in morning levels, occurs in a healthy individual, with a decline thereafter, reaching lowest basal level at bedtime (Pruessner et al., 1997). Thus, a flatter diurnal slope is regarded as an unhealthier profile (Adam et al., 2006).

Extensive research has been carried out on the association of cortisol levels and cognitive function but, to date, there is still little agreement on this relationship. Higher morning cortisol levels were reported to be associated with poorer cognitive function (Beluche et al., 2010; Venero et al., 2013) while other studies did not find the association (Gerritsen et al., 2011; Singh-Manoux et al., 2014; Stawski et al., 2011). However, recent studies demonstrated a contradictory result whereby lower morning levels were associated with poorer cognitive performance (Gerritsen et al., 2011; Stawski et al., 2011). Higher evening cortisol levels have also been associated with cognitive decline particularly in specific domains (Gerritsen et al., 2011; Gilpin et al., 2008; Li et al., 2006; Stawski et al., 2011) whereas another study reported no association (Venero et al., 2013). Impaired HPA axis reactivity assessed as higher daily mean of cortisol levels (Li et al., 2006) or flattened diurnal pattern (Fiocco et al., 2006; Kovach et al., 2011) were found to be associated with cognitive dysfunction.

Previous findings suggest that sex has an important role in the association between HPA axis activity and cognitive function, particularly among older people, with sex differences reported in some (Almela et al., 2012; Beluche et al., 2010; Seeman et al., 1997) but not all studies (Karlamangla et al., 2005). It has also been shown that cortisol responses differ in men and women, but whether the cortisol–cognition relationship mirrors the sex specific relationship of the general cortisol response is still uncertain. The association between cognitive status and cortisol secretion may also be influenced by other psychosocial, lifestyle and clinical factors which may impact cortisol levels such as age (Otte et al., 2005), alcohol intake (Badrick et al., 2008), sedentary lifestyle (Heaney et al., 2013), depressive symptoms (Potvin et al., 2013), and sleeping problems (Kumari et al., 2010). Thus, in this present investigation, these factors were considered as confounders in the association investigated between cortisol and cognition.

The conflicting evidence on the association of different cortisol secretion patterns with cognitive function needs clarification. To date, only three large, population-based, epidemiological studies with an age range of 35 to 85 years, have investigated this association and yielded mixed findings or applied only a specific cognitive domain (Gerritsen et al., 2011; Singh-Manoux et al., 2014; Stawski et al., 2011). Furthermore, there is a lack of large representative studies assessing the cortisol–cognition relationship with older adults, despite the fact that cognitive status is an important medical problem of the aged population. Thus, the major aims of this study were: (i) to examine the distribution of diurnal salivary cortisol across levels of cognitive status in a representative elderly population of KORA Age, (ii) to examine the association between salivary cortisol and cognitive function with sufficient adjustment for potential confounders and (iii) to assess the modifying role of sex on the association between cortisol and cognitive function.

2. Methods

2.1. Study setting and population

The KORA (Cooperative Health Research in the Region of Augsburg)-Age study was a follow-up study of the four MONICA/KORA Augsburg Surveys (Peters et al., 2011) which was conducted between November 2008 and November 2009. All participants, age 64 and older, were selected from a population based random sample ($N = 5991$) of the previous surveys (Survey 1–4, conducted between 1984 and 2001) with participation rates ranged between 67% and 79%. In total, 4127 people participated in a standardized telephone interview.

Table 1 Characteristics of KORA Age population stratified by cognitive status in *N* (%) or mean (SD) (*N* = 733).

	N	Probable dementia (4.5%, *N* = 33)		MCI (13.8%, *N* = 101)		Normal (81.7%, *N* = 599)		P^a	P^b
Sociodemographics									
Age	733	77.8	(±6.8)	77.5	(±5.6)	74.4	(±6.1)	**<0.0001**	
Male	375	23	(69.7)	71	(70.3)	281	(46.9)	**<0.0001**	
Female	358	10	(30.3)	30	(29.7)	318	(53.1)		
Low education	522	26	(78.8)	61	(60.4)	435	(72.6)	**0.03**	0.66
Risk factors									
Alcohol intake (g/day)	733	11.92	(±15.4)	13.2	(±15.0)	13.9	(±18.3)	0.66	**0.04**
Current smoker	31	1	(3.0)	4	(4.0)	26	(4.3)	0.93	0.99
Physically inactive	313	21	(63.6)	50	(49.5)	242	(40.4)	**0.01**	0.07
BMI (kg/m^2)	732	30	(±4.8)	28.5	(±4.1)	28.4	(±4.3)	0.13	0.09
Total cholesterol (mg/dl)	733	213.7	(±45.3)	199.7	(±36.7)	213.1	(±41.0)	**0.004**	0.48
HDL (mg/dl)	733	54.9	(±16.3)	52.5	(±13.3)	56.3	(±13.5)	0.005	0.35
LDL (mg/dl)	733	132.0	(±34.9)	121.7	(±32.9)	128.9	(±34.6)	0.07	0.85
Multimorbidity	731	32	(97.0)	90	(89.1)	535	(89.3)	0.08	0.30
Frail	24	1	(3.0)	9	(8.9)	14	(2.3)	**<0.0001**	**0.004**
Pre-frail	255	20	(60.6)	42	(41.6)	193	(32.2)		
Psychological factors									
Sleep disturbances	358	19	(5.3)	52	(14.5)	287	(80.2)	0.47	0.50
Depressive symptoms	43	5	(15.2)	5	(5.0)	33	(5.5)	0.07	0.06

[a] Differences across TICS categories, unadjusted *P*-value, Chisquare/Kruskal Walis.
[b] Age and sex adjusted *P*-value, GLM procedure.

Out of this group, a randomly drawn sample of 1079 participants additionally underwent extensive physical examinations including collection of blood samples, anthropometric examination, and a personal interview. For the current analysis, only participants who provided complete, plausible salivary samples (*N* = 772, saliva sampling rate of 72%, see Fig 1) were included. After exclusion of participants with missing data on cognitive score, the final data set for the present analysis consisted of 375 males and 358 females (age 65 to 90 years, mean age =74.9 years). In a drop-out analysis of the excluded participants there were no significant age and sex differences detected. The study was approved by the Ethics Committee of the Bavarian Medical Association and all participants provided a written informed consent.

2.2. Outcome: Telephone interview for cognitive status modified (TICS-m)

All study personnel who administered the TICS-m were specifically trained to administer the procedure. The TICS-m in German was administered according to published procedures as described elsewhere (Lacruz et al., 2013).

The TICS-m includes four domains: orientation; memory (registration, recent memory and delayed recall); attention/calculation; and language (semantic memory, comprehension and repetition). The total possible score for the TICS-m is 50 points. The following cut-offs were applied to education corrected TICS-m scores; ≤31 to separate subjects with mild cognitive impairment (MCI) from subjects with normal cognition, and ≤27 to separate subjects with probable dementia from subjects with MCI. The cut-offs were based on a previous validation study of education corrected TICS-m scores (Knopman et al., 2010).

2.3. Exposure: Salivary cortisol

Participants were individually instructed about the saliva sampling procedure and provided with additional detailed written information (Salivette® salivary sampling test kit). Three saliva samples were assessed; in the morning after awakening (M1), 30 min after awakening (M2), and in the late evening before bedtime (E). Cortisol levels were determined in duplicate using the Luminescence Immunoassay RE62011 (range 0.1—40 ng/ml, IBL) and the Victor Multilabel Plate Reader (Perkin Elmer). The intra-assay coefficients for cortisol analytes were less than 12.4% and the inter-assay coefficients were 10.2%.

2.4. Covariates

Sociodemographic variables included age and sex. Someone who smoked cigarettes regularly or occasionally was considered as a current smoker. The leisure time physical activity was assessed with two separate questions concerning leisure time sport activity in winter and in summer. In both questions, answer categories were Likert-scaled and ranged from ''1'' (''no sport'') to ''4'' (''more than two hours per week'') in winter and summer, respectively. Leisure time physical activity was assessed by two interview questions. Each participant was asked: ''How often do you carry out sports in the winter? How often do you carry out sports in the summer?'' Sports were broadly considered in the context of elderly participant activities and included both bicycle riding and going on walks. Answers were given on a four-level graded scale (no activity, irregularly about 1 h/week, regularly 1 h/week, and regularly >2 h/week) (Meisinger et al., 2005b). A participant was classified as physically active if

they regularly participated in sports during leisure time ≥1 h/week in either season. The variable was dichotomized into physically active or physically inactive. A person was rated as ''active'', if a person reported an hour or more of physical activity in either season. Multimorbidity was defined as the co-occurrence of more than two disease conditions based on the Charlson Comorbidity Index (Brandt et al., 1988). Frailty was assessed according to frailty criteria by Fried et al. (2001). Sleeping problems were assessed based on interview questions concerning the difficulty initiating and maintaining sleep, as well as sleeping duration (Meisinger et al., 2005a). Depressive symptoms were measured by the Geriatric Depression Scale (GDS-15; cut-off point >5 for mild or moderate depression) using the 15-item German version of the Geriatric Depression Scale (Sheikh and Yesavage, 1985).

2.5. Statistical analysis

Baseline descriptive analyses of demographic and clinical characteristics were stratified by cognitive status. In case of non-normality, tests were performed on log-transformed cortisol measurements. Cortisol awakening response was calculated based on the difference of M2 to M1 (CAR = M2 − M1) as suggested by previous studies (Kunz-Ebrecht et al., 2004; for review, see: Adam and Kumari, 2009; Clow et al., 2004) and the area under the curve with respect to ground (AUC_G) as suggested previously (Pruessner et al., 2003). The ratios of M1 to E (M1/E) and M2 to E (M2/E) were also calculated. LS (least squared) means (in mmol/l) and 95% confidence interval (CI) of cortisol measurements were calculated. Differences between groups (in age and sex adjusted models) and differences between men and women (adjusted for age) were tested with generalized linear model (GLM) procedures.

All significant covariates (age, sex, physical activity, smoking, frailty, sleeping problems and depressive symptoms) from univariate analysis of the study population and cortisol measures, with the outcome cognitive (TICS-m) score, were entered into a multiple linear regression model to assess the effect of cortisol levels on the risk of increased cognitive score. A sex-stratified multiple variable linear regression analysis was also considered. An additional multinomial logistic regression analysis was computed with a categorical cognitive status variable as the outcome, to distinguish the risk of being mildly cognitively impaired or probably demented (as opposed to having normal cognitive function). Multimorbidity and smoking were not significantly different between the cognitive status categories and therefore were not included in the model. Cortisol measurements were standardized by dividing each individual's unit value by the standard deviation of the sample, therefore odds ratios (OR) and 95% CI represent risk change per one standard deviation.

Analyses were performed using SAS statistical software, version 9.2 (SAS Institute, Cary, NC) and *P*-values of less than 0.05 were considered statistically significant. The STROBE checklist (STrengthening the Reporting of OBservational studies in Epidemiology) was applied in preparation of the manuscript.

3. Results

In the total population of 733 under investigation, 599 (81.7%) were participants with normal cognitive function. A subpopulation of 33 (4.5%) were qualified for the probable dementia group and 101 (13.8%) for the MCI category. Compared to participants with normal cognition, participants with probable dementia were more likely male, older, more physically inactive, and suffered from more comorbidities (Table 1). Frequencies and mean values of demographic and other variables are presented in Table 1 stratified by cognitive status. Sex differences were found in the current study population ($F = 45.76$, $P < 0.0001$), whereby men had lower scores (LS-mean = 34.54, SE = 0.23) than women (LS-mean = 36.78, SE = 0.24). TICS-m scores declined for all participants with increasing age ($F = 78.71$, $P < 0.0001$).

All cortisol measurements assessed and their association with cognitive status are displayed in Table 2. Participants with probable dementia and MCI had lower means of M1 ($P = 0.01$) and M2 ($P = 0.03$) salivary cortisol levels, and higher E ($P = 0.19$) levels compared to participants with normal cognition (Table 2). Lower CAR measurements were observed in participants with probable dementia as well as in MCI participants, while the highest were observed in healthy individuals. The association between cognitive function and both late evening cortisol and CAR measurements did not however reach statistical significance. In a sex stratified bivariate analysis (adjusted for age), no sex differences were observed in E and CAR measurements, while M2 levels, M1/E and M2/E ratios were significantly different in men and not in women as reported in Table 2.

Fig. 2 displays a sex-stratified finding of morning to evening cortisol ratios associated with cognitive status. Smaller M1/E ($P = 0.003$) and M2/E ($P = 0.007$) ratios were observed among probable dementia, and MCI participants compared to healthy individuals, in a dose–response manner for both men and women.

Multiple linear regression was employed to explore the association of cortisol levels and cognitive function with adjustment for age, sex, physical activity, alcohol intake, and frailty status. A continuous, one standard deviation (SD) increase in cortisol measurement was significantly associated with an increase in cognitive (TICS-m) score, which reflects a better cognitive function. In a crude model (adjusted for age and sex), a one SD increase in M1, M2, and both ratio (M1 to E and M2 to E) levels were all significantly associated with a higher TICS-m score (Table 3). A greater CAR (AUC_G) measurement was also associated with better cognitive function in the crude model, but failed to achieve statistical significance when additionally adjusted for alcohol intake, physical activity, sleep problems, depressive symptoms, and frailty status. A fully adjusted model (Table 3) revealed that participants with higher morning to evening ratios (both M1/E and M2/E) had increased odds of having higher TICS-m score, and thus, better cognitive performance (M1/E: OR = 1.50, 95% CI = 1.08–2.07, $P = 0.01$; M2/E: 1.41, 1.01–1.95, 0.04 per one SD increase). In other words, those participants with lower cortisol in the morning and higher evening levels were more likely to have poorer cognitive status.

Table 2 Various cortisol measurements (least squared (LS) mean, mmol/l, 95% CI) stratified by cognitive status ($N = 733$).

	Probable dementia	MCI	Normal	P^a	♂	♀
log M1M1	1.25 (1.04–1.46)	1.22 (1.10–1.35)	1.36 (1.32–1.41)	**0.01**	0.12	0.18
log M2	1.48 (1.27–1.69)	1.51 (1.39–1.63)	1.64 (1.59–1.69)	**0.03**	**0.02**	0.58
log E	−0.18 (−0.41–0.06)	−0.28 (−0.41 to −0.41)	−0.37 (−0.42 to −0.31)	0.19	0.06	0.54
log (M1/E)	1.43 (1.15–1.71)	1.50 (1.34–1.66)	1.73 (1.66–1.80)	**0.003**	**0.006**	0.13
log (M2/E)	1.66 (1.37–1.95)	1.79 (1.62–1.95)	2.00 (1.93–2.07)	**0.007**	**0.001**	0.35
CAR	1.09 (0.07–2.12)	1.31 (0.72–1.90)	1.38 (1.14–1.62)	0.77	0.30	0.64
CAR (AUC_G)	136.25 (112.55–159.96)	146.46 (132.72–160.20)	156.29 (150.73–161.85)	0.09	0.21	0.32

Abbreviations: M1 = morning after waking; M2 = 30 min after waking; E = evening; CAR = cortisol awakening response; AUCG = area under the curve with respect to ground; M1/E = M1 to E ratio; M2/E = M2 to E ratio; MCI = mild cognitive impairment.
[a] Differences between cognitive status (age and sex adjusted by GLM procedure).

Interestingly, each incremental SD increase in both M1 and M2 morning cortisol levels was associated with increased odds of better cognitive function (M1: OR = 1.42, CI = 1.03–1.95, $P = 0.03$; M2 = 1.32, 0.95–1.81, 0.10) while increases in late evening cortisol levels were associated with a reduced odds of better cognitive status (0.84, 0.61–1.17, 0.30). In the final model, M1, M1/E and M2/E ratios achieved statistical significance. In a separate multinomial regression analysis, both ratios (M1/E and M2/E) were significantly associated with mild cognitive impairment (MCI) (see Supplementary Table 1). In a sex-stratified analysis, M1, M2, E levels and both ratios of M1/E and M2/E levels were significantly associated with cognition in men and not women (Table 4). In fully adjusted model, only the M1/E and M2/E ratios were significantly associated with increased odds of better cognition (M1/E: OR = 1.94, 1.24–3.02, 0.004; M2/E = 1.74, 1.12–2.71, 0.01) in men.

4. Discussion

This is the first community-based study with an older population which demonstrates that cognitive impairment is associated with a reduced ratio of morning to evening

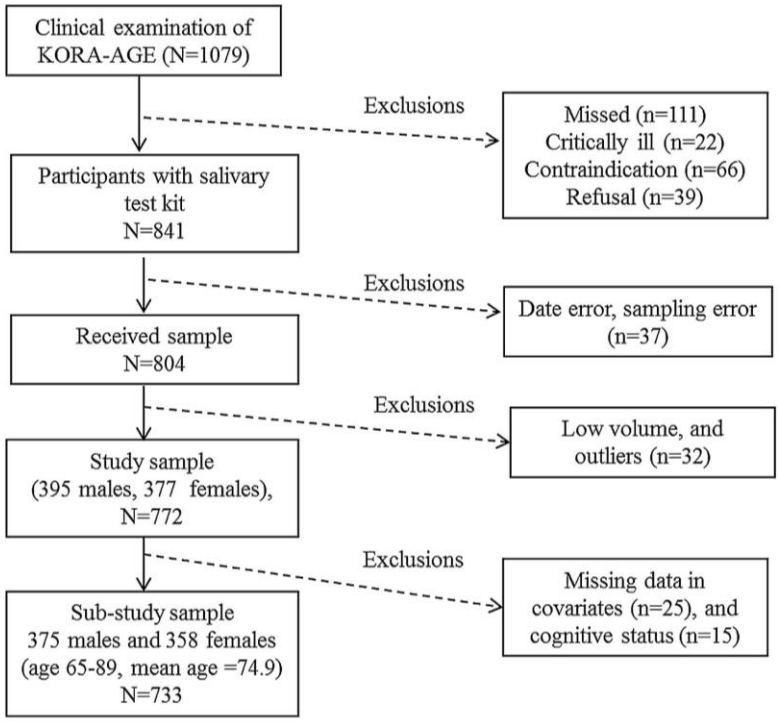

Fig. 1 Flow chart of the cross-sectional study design derived from the KORA (Cooperative Health Research in the Augsburg Region, Germany) Age-1 Study (2008–2009).

Table 3 Multivariable adjusted odds ratio (OR), 95% confidence intervals and *P*-values for the association of various cortisol measurements and cognitive status (TICS-m score corrected for education years) ($N = 733$).

Cortisol measurements	Crude model[a]			Full model[b]		
	OR	(95% CI)	*P*-value	OR	(95% CI)	*P*-value
log M1	1.52	(1.10–2.10)	**0.01**	1.42	(1.03–1.95)	**0.03**
log M2	1.45	(1.05–2.00)	**0.03**	1.31	(0.95–1.81)	0.10
log E	0.80	(0.58–1.11)	0.19	0.84	(0.61–1.17)	0.30
log (M1/E)	1.64	(1.19–2.27)	**0.003**	1.50	(1.08–2.07)	**0.01**
log (M2/E)	1.57	(1.13–2.19)	**0.01**	1.41	(1.01–1.95)	**0.04**
CAR	1.05	(0.76–1.46)	0.77	0.98	(0.71–1.36)	0.92
CAR (AUC_G)	1.39	(1.00–1.92)	**0.05**	1.27	(0.92–1.75)	0.15

[a] Crude model: age and sex adjusted.
[b] Full model: age, sex, alcohol intake, physical activity, sleep problems, depressive symptoms, and frailty status. Odds ratio (OR) is for a 1 standard deviation (SD) increase of cortisol level (SD of log M1 = 0.61, log M2 = 0.62, log A = 0.69, log (M1/E) = 0.83, log (M2/E) = 0.86, CAR = 2.99, CAR(AUC_G) = 68.85). Abbreviations: M1 = morning after waking, M2 = 30 min after waking, E = evening, CAR = cortisol awakening response, AUC_G = area under the curve with respect to ground.

cortisol levels. The reduced or lower ratio reflects a diminished diurnal variability of morning to evening levels while a larger ratio is indicative of a wider diurnal span, and is observed in participants with a better cognitive performance. A smaller ratio which is also comparable to a flat diurnal pattern, is associated with poorer cognition as demonstrated in previous studies with cognition and diurnal slope measurements (Beluche et al., 2010; Fiocco et al., 2006; Gerritsen et al., 2011; Kovach et al., 2011). Thus, the clearest findings in this study are not derived from absolute values of cortisol per se, but rather from measurements that assess dynamic changes, from the nadir at night to highest levels in the morning.

4.1. Blunted cortisol response is associated with poorer cognitive function

Healthy adults have substantially increased cortisol levels in the morning that then decrease to the lowest level in the evening. A highlight of our study is the inclusion of participants with both MCI and probable dementia in which we showed that a healthy cortisol profile is compromised in these participants who have lower morning cortisol levels and fail to achieve low levels in the evening. This cortisol–cognition relationship was statistically significant in men but not in women. Although a similar dose–response pattern was observed in both sexes, the association failed

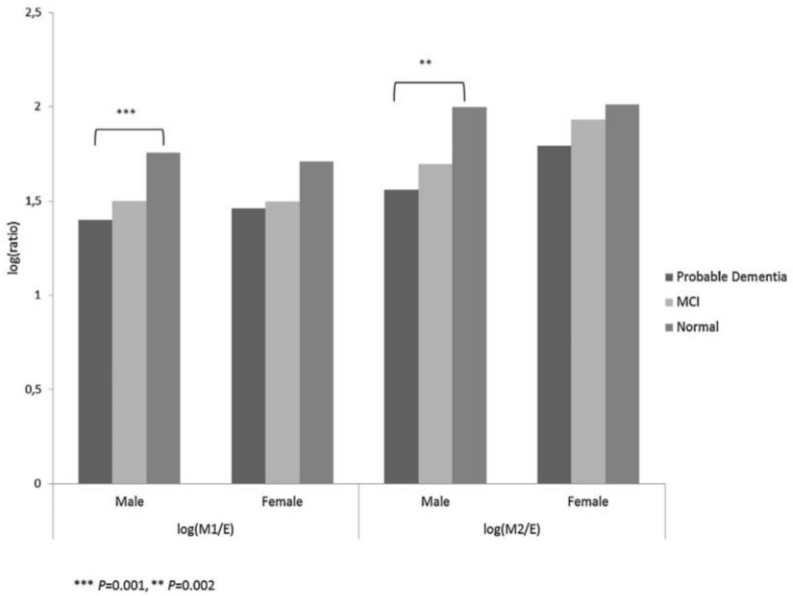

Fig. 2 Ratio of morning to evening cortisol levels (least squared (LS) means, 95% CI) by cognitive status, stratified by sex (adjusted for age).

Table 4 Multivariable adjusted odds ratio (OR), 95% confidence intervals and *P*-values for the association of various cortisol measurements and cognitive status (TICS-m score corrected for education years) stratified by sex ($N = 733$).

Model 1	Male			Female		
	OR	(95% CI)	*P*-value	OR	(95% CI)	*P*-value
log M1	1.71	(1.08–2.73)	0.02	1.37	(0.87–2.15)	0.18
log M2	1.60	(1.00–2.55)	0.05	1.32	(0.84–2.09)	0.23
log E	0.62	(0.40–0.96)	0.03	1.09	(0.67–1.78)	0.73
log (M1/E)	2.21	(1.42–3.45)	0.001	1.21	(0.75–1.94)	0.44
log (M2/E)	2.03	(1.31–3.17)	0.002	1.18	(0.72–1.93)	0.51
CAR	1.60	(0.95–2.69)	0.08	1.33	(0.73–2.42)	0.36
CAR(AUC_G)	1.41	(0.89–2.23)	0.14	1.36	(0.86–2.16)	0.19
Model 2						
log M1	1.59	(1.00–2.51)	0.05	1.26	(0.80–1.98)	0.32
log M2	1.42	(0.89–2.26)	0.14	1.23	(0.78–1.94)	0.38
log E	0.68	(0.44–1.05)	0.09	1.12	(0.68–1.82)	0.66
log (M1/E)	1.94	(1.24–3.02)	0.004	1.11	(0.69–1.78)	0.67
log (M2/E)	1.74	(1.12–2.71)	0.01	1.09	(0.67–1.77)	0.74
CAR	1.43	(0.85–2.42)	0.18	1.17	(0.64–2.14)	0.62
CAR(AUC_G)	1.30	(0.83–2.04)	0.25	1.25	(0.78–1.98)	0.35

Model 1: adjusted for age. Model 2 (full model): adjusted for age, physical activity, alcohol intake, frailty status, sleep problems, depressive symptoms. Odds ratio (OR) is for a 1 standard deviation (SD) increase of cortisol level (SD of log M1 = 0.61, log M2 = 0.62, log E = 0.69, log (M1/E) = 0.83, log (M2/E) = 0.86, CAR = 2.99, CAR(AUC_G) = 68.85). Abbreviations: M1 = morning after waking; M2 = 30 min after waking; E = evening; CAR = cortisol awakening response; AUC_G = area under the curve with respect to ground.

to achieve a statistically discernible difference in women. The insignificant finding in women may not reflect the biological differences but may rather be due to the low number of women in the cognitively impaired group. Additionally, the association between cortisol and cognitive task performance in women may have been confounded by other unknown factors.

To the best of our knowledge, we are the first to apply the ratio concept for the assessment of the association between cortisol and cognition. There are however three epidemiological studies which are comparable to this present investigation (Gerritsen et al., 2011; Singh-Manoux et al., 2014; Stawski et al., 2011). These studies have presented findings on both morning and evening cortisol levels, but did not apply the ratio construct. The Longitudinal Aging Study Amsterdam (LASA) ($N = 911$, mean age 74.5 ± 7.2 years) confirmed the finding for a specific memory domain only among APOE-ε4 carriers but did not present sex-specific findings (Gerritsen et al., 2011) whereas the MIDUS study included a wide age range (33–84 years) and showed a significant positive relationship with executive functioning (Stawski et al., 2011). These studies demonstrated that lower morning and higher evening cortisol levels as well as a flat diurnal slope pattern was associated with cognitive impairment. In contrast, the Whitehall II study ($N = 3229$, mean age 61 years) revealed no significant longitudinal association between diurnal cortisol patterns and cognition (Singh-Manoux et al., 2014). Furthermore, there are other clinical studies that examined these relationships but yielded mixed findings with most studies reporting higher morning cortisol levels. In addition, some studies focused on specific study populations, for example, among APOE-ε4 carriers and non-carriers (Gerritsen et al., 2011; Lara et al., 2013; Lee et al., 2008), MCI subjects (Lind et al., 2007; Venero et al., 2013), and diabetic patients (Reynolds et al., 2010). Thus, these previously mentioned studies were not generalizable to the older general population.

4.2. Cortisol secretion in the morning and the evening

It is well established that the general cortisol circadian pattern exhibits a particular hypersecretion of cortisol levels in the morning followed by a steady decrease in secretion levels throughout the day reaching basal level at night. A deviation from these levels may reflect an individual's unhealthy state. The present study demonstrated that lower morning cortisol is associated with poorer cognitive performance (OR = 1.42, 95% CI = 1.03–1.95). In direct contrast to our findings, higher morning cortisol levels have been associated with diminished global cognitive function (MacLullich et al., 2005) as well as with specific domains (e.g. verbal fluency (Beluche et al., 2010; Geoffroy et al., 2012), delayed recall and processing speed (MacLullich et al., 2005)). However, there is also evidence of diminished morning cortisol levels that are associated with cognitive impairment in the general population (Gerritsen et al., 2011; Stawski et al., 2011) and among participants without anxiety or depression (Potvin et al., 2013). Thus, these studies contribute to more conflicting evidence which opens up this issue for further research.

Failure to achieve low basal evening levels has also been associated with poorer cognitive function as also evidenced in previous epidemiological (Gerritsen et al., 2011; Stawski et al., 2011) and clinical studies (Gilpin et al., 2008; Li

et al., 2006). The late evening measurement most reliably demonstrates age-related elevations of cortisol levels, as it represent the crucial phase of achieving the circadian nadir (lowest value of the oscillatory function) (Li et al., 2006). Thus, in addition to the morning cortisol measurement, the late evening cortisol is also important to consider when assessing the association between cortisol and cognitive function, as evidenced by our significant findings with the M1/E ratios and cognition.

4.3. The modifying role of sex

Only a minority of studies have reported on sex differences in the association of cortisol levels and cognitive function. In women, higher evening cortisol was associated with greater decline in reasoning (Singh-Manoux et al., 2014), greater urinary cortisol excretion was associated with memory decline (Seeman et al., 1997), and high morning as well as a flatter cortisol pattern was associated with poorer verbal fluency and visual memory (Beluche et al., 2010). In men, elevated CAR was associated with poorer memory (Almela et al., 2012) and a flatter diurnal pattern in poorer visuo-spatial and visual memory performance (Beluche et al., 2010).

The associations of lower morning, as well as lower morning to evening cortisol level ratios, with poorer cognitive function in men have not been previously reported. In this present investigation, an impaired cortisol regulation was significantly associated with poorer cognition in men but not women. However, fewer (30%) female participants were categorized in the cognitively impaired group which may have decreased the power of the subgroup analysis and thus limits the interpretation of the findings. However, this sex-specific finding of dysregulated cortisol secretion observed in men and not women corroborates a previous finding of an fMRI study in which stress was associated with asymmetric prefrontal activity and cortisol variation in men, whereas in women, stress was associated with limbic activation and less correlated with cortisol (Wang et al., 2007). Men also display a higher cortisol stress response compared to women in acute stress exposure in laboratory settings (For review, see Kudielka and Kirschbaum, 2005). Furthermore, Hogervorst et al. (2004) also demonstrated that men performed worse in the memory task (immediate and delayed memory) of TICS-m compared to women which may also influence the observed associations in men.

4.4. Cortisol levels and MCI

In a dose—response manner, participants with mild cognitive impairment (MCI) and probable dementia had lower morning to evening cortisol ratios in comparison to subjects with normal cognition. The strength of the association between the cortisol ratio and cognition was strongest in participants with probable dementia, but less pronounced in the MCI subjects, thus supporting the dose—response relationship. The present investigation also provided data on the differences between dementia and MCI which adds to current, contradicting literature. Previous studies have reported that MCI individuals showed increased cortisol levels compared to cognitively healthy subjects (Lee et al., 2007; Lind et al., 2007; Lupien et al., 1998) while others found no significant differences (Wolf et al., 2002). This is particularly important as MCI is an established risk factor for cognitive decline (Peavy et al., 2007). Subtypes of MCI have been associated with morphological and functional changes in certain brain structures (Qiu et al., 2009) which may modulate the HPA axis regulation, thus affecting circadian cortisol secretion (McEwen, 2008).

4.5. Study strengths and limitations

The present study collected salivary cortisol samples from a large group of men and women with a high response rate and a very strict quality assessment which were strengths of this present study. In this study, we applied the morning and evening ratio which represents a reliable assessment of cortisol secretion rhythm in a 24 h period. High participation sampling rates were achieved through telephone calls, despite some individuals having MCI or probable dementia.

KORA Age also has the advantage of using the TICS-m measurement that is a feasible tool to screen for cognitive impairment in large-scale epidemiological settings with older participants (Lacruz et al., 2013). The distribution of the TICS-m has been shown to have a more symmetric and normal-shaped distribution than the commonly used Mini-Mental State Examination (MMSE), suggesting that TICS-m is less subject to the ceiling effect that habitually limits MMSE, thus making TICS-m more sensitive than MMSE (Lacruz et al., 2013). Although the categorizations of MCI and probable dementia are based on a single cognitive test score, the TICS-m have been shown to be sensitive for detecting early cognitive impairment and useful in large study settings (Lacruz et al., 2013). According to published cut-offs (Knopman et al., 2010), 6% of the study participants were categorized as having probable dementia (Lacruz et al., 2013) which is also comparable to another population-based study where dementia was shown to affect about 6.4% of European subjects over the age of 65 years (Lobo et al., 2000).

The limitations of this study were one sampling day of cortisol and limited numbers of salivary samples (3 samples) were collected. More intensive sampling of cortisol throughout the day would better represent the diurnal cortisol secretion pattern (Hellhammer et al., 2007). It was suggested that sampling of cortisol is needed to be taken every 15 min after awakening to capture a more accurate morning cortisol rise (Hellhammer et al., 2007). Similarly, an hourly or bihourly sampling was suggested to provide more information for the dynamics of the diurnal pattern (Cohen et al., 2006). However, with limited cortisol sampling times, our study could demonstrate the association of the simple morning to evening ratio with cognitive function which facilitates an improved understanding of the dynamics of the cortisol daily secretion with cognition in the elderly. Another limitation of this study is the small sample of participants with probable dementia which potentially explains the non-significant findings in the cognitively impaired individuals.

The present study has no available data for APOE-ε4 polymorphism. This may be a limitation because previous studies have suggested the modifying role of APOE-ε4 on the association between cortisol levels and cognition (Gerritsen et al., 2011; Lee et al., 2008). However, recent evidence does not

support the modifying role of APOE-ε4 in this relationship (Fiocco et al., 2008; Lara et al., 2013).

The use of the simple morning to evening cortisol level ratios is relatively unexplored. The daily dynamics of cortisol secretion, as an indication of an individual's ability to adjust from the highest level in the morning to the lowest, basal level at night, were clearly captured in the ratio concept. Thus, it is important to assess the ratio of the morning and evening measurements and not only the absolute values, in order to ascertain an individual's balance between morning and evening cortisol. Further investigation is needed to determine whether the morning to evening balance is a clinically relevant therapeutic target, and whether there is a gender effect in the cortisol—cognition relationship in old age. Since the nature of cross-sectional studies cannot infer causality, a prospective analysis of the study population is warranted.

A blunted diurnal cortisol pattern, as indicated by a low morning to evening ratio, appears to be a sensitive measure of decreased physiologic resilience that leads to diminished cognition. Blunted cortisol secretion may indicate increased allostatic load and a risk for chronic disease progression as evidenced recently by an association with prefrail and frail states in the same study population (Johar et al., 2014). Biological evidence for the deleterious effects of dysregulated HPA axis can be observed using numerous markers of brain health, including atrophy, deactivation, and cell death in the hippocampus (McEwen, 1999), a region important to memory and risk for Alzheimer's disease. Thus, a smaller morning to evening ratio may reflect a dysregulated HPA axis, which imposes a significant physiological burden on multiple, interrelated body systems. Implications of these findings may allow for earlier detection of physical and cognitive decline, particularly in patients in early stages of decline that may still positively respond to therapeutic intervention.

5. Conclusion

This study presents evidence for the relationship between cognitive function and cortisol profiles which suggests that blunted cortisol reactivity is associated with cognitive impairment among older men and women. Lower morning cortisol and higher morning to evening ratios are associated with poorer cognitive function. Thus, the morning to evening cortisol ratio may provide crucial information on the underlying mechanism of cognitive decline. Furthermore, the ability to identify individuals with MCI requires more attention as these elderly people, who are most at risk for progressing to dementia, are a critical target for intervention strategies.

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Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.10.011.

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Supplementary table 1: Associations of various cortisol measurements at specific time-points with cognitive impairment (Reference=normal) (N=733)

Cortisol Measurements	MCI vs. Normal		Probable Dementia vs. Normal	
	OR, 95% CI	*p*-value	OR, 95% CI	*p*-value
M1	0.82 (0.67-1.00)	0.05	0.87 (0.63-1.22)	0.42
M2	0.84 (0.68-1.03)	0.09	0.85 (0.62-1.18)	0.33
E	1.12 (0.90-1.38)	0.32	1.29 (0.92-1.81)	0.13
M1/E	0.74 (0.57-0.96)	**0.02**	0.66 (0.43-1.02)	0.06
M2/E	0.77 (0.60-0.99)	**0.04**	0.67 (0.45-1.00)	0.05
CAR	0.98 (0.53-1.82)	0.95	0.85 (0.29-2.49)	0.77

adjusted for age, sex, physical activity, alcohol intake, frailty, sleep and depressive symptoms with the outcome of cognitive status as measured by TICS-m (adjusted by education)

Odds ratio (OR) is for a 1 standard deviation (SD) increase of cortisol level (SD of logM1 = 0.61, logM2 = 0.62, logA = 0.69, log(M1/E) = 0.83, log(M2/E) = 0.86, CAR = 2.99)

Abbreviations: M1=Morning after waking, M2=30 minutes after waking, E=Evening, CAR=Cortisol Awakening Response, MCI = Mild Cognitive Impairment

8. Paper 3: Impaired Sleep Predicts Cognitive Decline in Old People

Original title:	Impaired Sleep Predicts Cognitive Decline in Old People: Findings from the Prospective KORA Age Study
Authors:	Hamimatunnisa Johar; Rasmila Kawan; Rebecca Thwing Emeny, Karl-Heinz Ladwig *Hamimatunnisa Johar and Rasmila Kawan shared the first authorship.
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SLEEP AND AGING

Impaired Sleep Predicts Cognitive Decline in Old People: Findings from the Prospective KORA Age Study

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Study Objectives: To investigate the association between sleep-related characteristics and cognitive change over 3 years of follow up in an aged population.
Methods: Sleep characteristics and covariates were assessed at baseline in a standardized interview and clinical examination of the population-based KORA Age Study (n = 740, mean age = 75 years). Cognitive score (determined by telephone interview for cognitive status, TICS-m) was recorded at baseline and 3 years later.
Results: At baseline, 82.83% (n = 613) of participants had normal cognitive status, 13.51% (n = 100) were classified with mild cognitive impairment (MCI), and 3.64% (n = 27) with probable dementia. The effect of three distinct patterns of poor sleep (difficulties initiating [DIS] or maintaining sleep [DMS], daytime sleepiness [DS] or sleep duration) were considered on a change in cognitive score with adjustments for potential confounders in generalized linear regression models. Cognitive decline was more pronounced in individuals with DMS compared to those with no DMS (β = 1.33, 95% CI = 0.41–2.24, P < 0.001). However, the predictive power of DMS was only significant in individuals with normal cognition and not impaired subjects at baseline. Prolonged sleep duration increased the risk for cognitive decline in cognitively impaired elderly (β = 1.86, 95% CI = 0.15–3.57, P = 0.03). Other sleep characteristics (DIS and DS) were not significantly associated with cognitive decline.
Conclusions: DMS and long sleep duration were associated with cognitive decline in normal and cognitively impaired elderly, respectively. The identification of impaired sleep quality may offer intervention strategies to deter cognitive decline in the elderly with normal cognitive function.
Keywords: elderly, sleep, cognitive decline, dementia
Citation: Johar H, Kawan R, Emeny RT, Ladwig KH. Impaired sleep predicts cognitive decline in old people: findings from the prospective KORA age study. *SLEEP* 2016;39(1):XXX–XXX.

Significance
Sleep-related characteristics are associated with an increased risk of cognitive decline. These characteristics are potentially modifiable risk factors that may offer strategies to improve cognitive function. However, prospective studies on the association between sleep-related characteristics and cognitive decline are sparse. No efforts to distinguish between cognitively intact and impaired individuals have been reported. We investigated the association between sleep-related characteristics and cognitive decline in older adults over 3 years of follow up and found that the individuals with normal cognition at baseline were at risk if they experienced difficulties maintaining sleep. This could be an early warning sign of increased risk of cognitive decline and should receive particular attention.

INTRODUCTION

Cognitive decline is a common problem in aging populations and is a major health concern that may confer serious challenges on daily activities of personal life and family functioning.[1] Thus, it is important to identify possible predictors of cognitive decline in order to develop effective preventive measures.

Recently, the sleep-cognition relationship has gained considerable attention in aging studies. As listed in Table 1, impaired sleep patterns under investigations have included prolonged or short sleep duration, excessive daytime sleepiness, snoring, as well as difficulties initiating (DIS) and maintaining sleep (DMS). Previous cross-sectional studies on the relation of sleep characteristics to cognition have predominantly focused on sleep duration and excessive daytime sleepiness, with most findings supporting an association between both self-reported long sleep duration and excessive daytime sleepiness with lower levels of cognitive function.[2–6] However, conflicting evidence was presented in a recent large scale cross-sectional study that emphasized the association of short sleep duration on cognitive decline.[4] In longitudinal studies, problems in initiating and maintaining sleep, excessive daytime sleepiness, and short and prolonged sleep duration were all associated with cognitive decline.[7–15] In contrast, evidence from other longitudinal studies failed to confirm a significant association between cognitive impairment and either sleep difficulties (DIS and DMS),[10,16] long sleep duration, or snoring.[16] Furthermore, several shortcomings of the previous published work includes study samples that are restricted to either male[7,9] or female[13,16] populations. To date, studies which reported significant associations between cognitive decline and problems maintaining sleep in both men and women remain sparse. Furthermore, none of these studies considered the influence of baseline cognitive status on the adverse effect of sleep on cognitive change.

Based on these mixed findings, the evidence for an association of various patterns of poor sleep and cognitive decline is still inconclusive. Despite extensive scientific work in sleep-cognition research in the recent years, there is still an unsatisfying research gap that demands further studies. Therefore, we aimed to investigate whether major patterns of poor sleep (difficulties in initiating sleep [DIS], difficulties in maintaining sleep [DMS] or both DIS and DMS, daytime sleepiness, and sleep duration) were associated with cognitive decline in a population-based sample of older adults (64–94 years) over 3 years of follow-up. Among the various risk factors of cognitive decline, baseline cognitive status has been shown to be one of the most important predictors. Several studies have demonstrated the predictive value of baseline cognitive function for further

Table 1—Prospective, population-based studies investigating sleep characteristics associations with cognitive decline.

References	Settings	n	Men/ Women (%)	Age (range/ mean)	Sleep Characteristics	Sleep Assessment	Cognitive Test	Main Findings	Confounders
CROSS-SECTIONAL STUDIES									
Ramos et al. (2013)	The Northern Manhattan Study (NOMAS)	2,266	39/61	75 ± 9	sleep duration, sleep disordered breathing, daytime sleepiness	Subjective	MMSE	Long sleep duration was associated with worse MMSE performance (or lower cognitive function).	age, sex, race-ethnicity, education, insurance status
Blackwell et al. (2006)	Community dwelling women	2,932	0/100	≥ 65	total sleep time, sleep efficiency, sleep latency, wake after sleep onset, total nap time	Objective	MMSE	Poor sleep was associated with impaired cognitive function in older women.	age, race, depression, education, health status, hypertension, smoking, alcohol use, physical activity
Blackwell et al.(2011)	The MrOS Sleep Study	3,132	100/0	≥ 65	total sleep time, sleep efficiency, wake after sleep onset, poor sleep, excessive daytime sleepiness	Subjective and objective	MMSE	Short sleep duration and excessive daytime sleepiness was not significantly associated with cognitive function.	age, race, BMI, co-morbidities, education, smoking, alcohol use, physical activity, depression
Merlino et al.(2010)	Community dwelling adults	750	39/61	≥ 65	Insomnia, snoring, sleep apneas, restless leg, sleep walking, daytime sleepiness	Subjective	MMSE	Daytime sleepiness was associated with dementia and cognitive decline	age, education, nervous system diseases, drugs
Elwood et al.(2010)	The Caerphilly Cohort Study		100/0	60–74	Insomnia, restless legs, snoring, sleep apnea, daytime sleepiness	Subjective		Sleep disturbance and daytime sleepiness was associated with vascular dementia.	age, social class, smoking, alcohol use, BMI, chest pain, drugs
PROSPECTIVE STUDIES									
Blackwell et al. (2014)	The MrOS Study	2,822	100/0	76 ± 5.3	sleep time, sleep onset, wake episodes	Subjective and objective	MMSE	Reduced sleep efficiency, DIS, DMS, poor sleep quality were associated with cognitive decline in older men.	depressive symptoms, comorbidities, medication use
Cricco et al. (2001)	The EPESE Study	6,444	75/35	> 65	symptoms of insomnia	Subjective	SPMSQ	Chronic Insomnia was associated with incident cognitive decline in older men	age, race, education, smoking, alcohol consumption
Foley et al. (2001)	Honolulu Asia Aging Study (HAAS)	2,346	100/0	71–93	daytime sleepiness	Subjective	CASI	Daytime sleepiness was associated with 3-Year incident Dementia and cognitive decline in men.	age, education, hours of sleep, daytime napping, heart diseases
Jelicic et al. (2002)	Maastricht Ageing Study (MAAS)	838	52/48	> 50	sleep problems (falling asleep, waking up too early, disturbed sleep)	Subjective	MMSE	Subjective sleep problems was associated with cognitive decline	age, sex, cognitive function at baseline, education
Tworoger et al. (2006)	The NHS Cohort	1,844	0/100	70–81	sleep duration, difficulty sleeping, snoring	Subjective	TICS	Sleep duration, difficulty sleeping and snoring were not significantly associated with cognitive decline in women	age, education, smoking, physical activity, alcohol consumption, hypertension
Benito-leon et al. (2009)	The NEDICES Study	3,286	42/57	73	sleep duration	Subjective	MMSE	Long sleep duration was associated with risk for dementia	baseline age, educational level, smoking, alcohol consumption
Loerbroks et. al.(2009)	The HeiDE Study	4,010	0/100	> 70	sleep duration	Subjective	TICS	Long sleep duration was significantly associated with cognitive impairment	hypertension, stroke, diabetes
Jaussent et al. (2012)	The French Three-City Study	4,894	43/57	> 65	excessive daytime sleepiness, difficulty in maintaining sleep	Subjective	MMSE	Excessive daytime sleepiness was associated with cognitive decline whereas poor sleep quality, DIS and early morning awakening were not	age ,sex, BMI, sleep medicines
Keage et al. (2012)	Cognitive Function and Ageing study	2,041	47/53	> 65	maintenance and onset of sleep	Subjective	MMSE	Napping at baseline, short sleep duration and excessive daytime sleepiness were associated with cognitive decline.	age, sex, BMI, education
Potvin et al. (2012)	ESA Study (Survey on Elder's Health	1,664	30/70	65–96	sleep duration, sleep quality	Subjective	MMSE	In men, short sleep duration and sleep efficiency was associated with cognitive impairment. In women, sleep disturbance and long sleep duration were associated with cognitive decline.	education, anxiety, chronic diseases, cardiovascular conditions
Benito-leon et al. (2013)	The NEDICES Study	2,715	43/57	73	sleep duration	Subjective	MMSE	Long sleep duration was associated with cognitive decline.	baseline age, gender, education, geographical area, depression
Virta et al. (2013)	Older Finnish Twin Cohort	2,336	52/48	> 65	sleep duration, sleep quality	Subjective	TICS	Short sleep duration, poor sleep quality and use of medications were associated with cognitive decline.	age, sex, education, follow up time
Devore et al. (2014)	Prospective Nurses' Health Study Cohort	15,385	0/100	> 70	sleep duration	Subjective	TICS	Long sleep duration was associated with cognitive decline.	age, education, smoking, physical activity, alcohol intake

CASI, cognitive abilities screening Instrument, SPMSQ, Short Portable Mental Status Questionnaire, MMSE, mini-mental status examination, TICS, telephone interview for cognitive status.

decline which provided the rationale for adjusting for baseline values in models of cognitive change in the current study.[17,18] Furthermore, the association between sleep and cognitive function was considered in the presence of potential confounders which have been acknowledged as risk factors of cognitive decline: age, sex, baseline cognitive score, smoking status, alcohol consumption, physical activity, depressive symptoms, hypertension, and somatic comorbidities.[9,10,12,13,15,19–21]

METHODS

Design and Setting and Participants

The data from the present investigation stem from the KORA-Age Study which is a population-based study that aimed to examine the prevalence and determinants of functioning, multi-morbidity and successful aging in men and women aged 64 to 94.[22,23] KORA-Age is a follow-up examination of

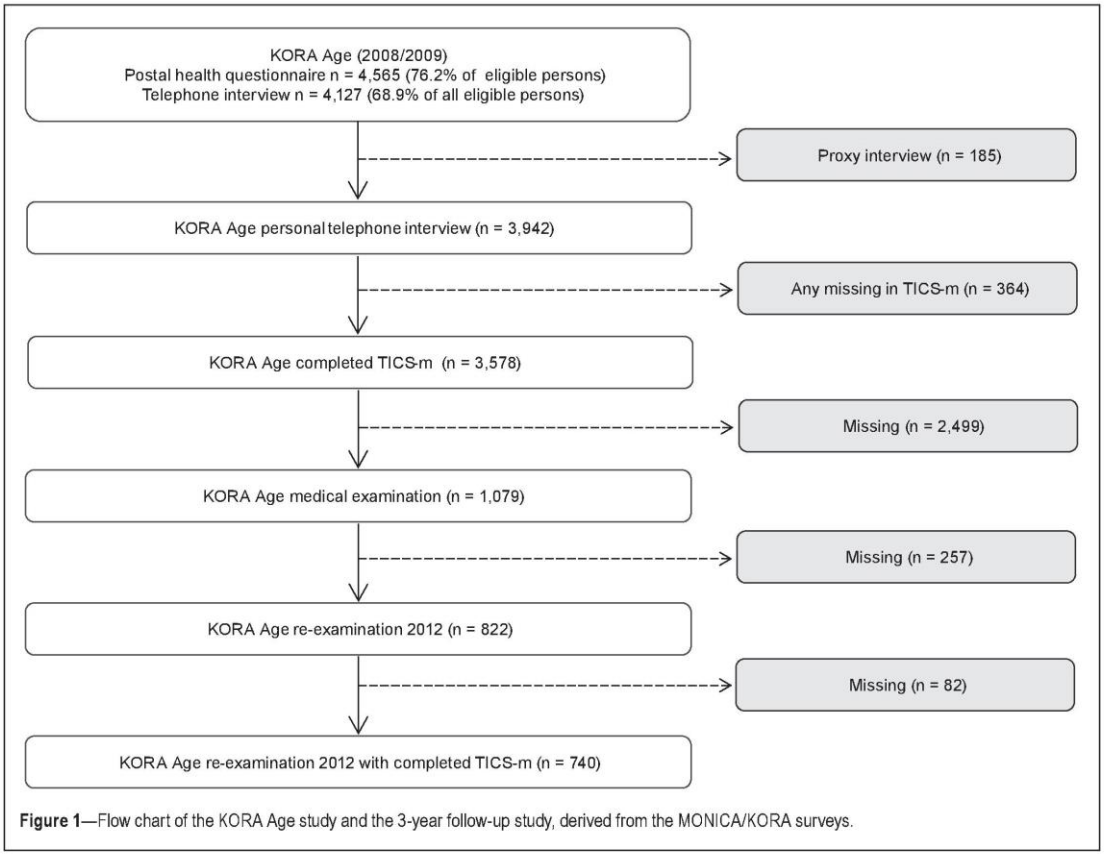

Figure 1—Flow chart of the KORA Age study and the 3-year follow-up study, derived from the MONICA/KORA surveys.

the participants (≥ 64 years) of the previous surveys of the MONICA/KORA (MONItoring of trends and determinants in CArdiovascular disease/Cooperative Research in the Region Augsburg) Study, which has collected data since 1984. Participants live in the city of Augsburg or its two surrounding counties in southern Germany.

In 2009, a clinical examination was performed in 1,079 participants in a gender- and age-stratified random sample of the MONICA cohort. In 2012, a re-examination was performed in 822 persons (84.3% of all eligible persons). Both baseline and follow-up surveys included a telephone and personal interview and a physical examination which assessed somatic and mental health related multi-morbidity. A total of 740 study participants (men = 49 %, n = 366, women 51%, n = 374, mean age = 75 years (range 64–94 years) with complete data on baseline covariates and cognitive status as well as cognitive status at follow-up were included in the study (Figure 1). In a drop-out analysis of the excluded participants, there were no significant age and sex differences observed (data not shown).

Quality control in the KORA Age study was assured by extensive operation manuals, training and certification of interview and examination personnel, a pilot study well in advance of the main study, and an external quality assurance audit. Internal quality control was performed to regularly monitor all relevant aspects of data acquisition. Written informed consent was obtained from each study participant and the study was approved by the ethics committee of the Bavarian Medical Association.

Endpoint: Cognitive Status

Cognitive status was measured by using the German language version of the Telephone Interview for Cognitive Status modified (TICS-m) which includes adjustment for years of education.[24] The TICS-m in German was administered according to published procedures.[24] The TICS-m includes 2 additional tasks: immediate and delayed verbal recall. The TICS-m contains the following items: (i) name, date, age and phone number (9 points); (ii) counting backward (2 points); (iii) first, a 10-word list learning exercise and then a delayed recall of that word list (20 points); (iv) serial sevens (5 points); (v) responsive naming (4 points); (vi) repetition (2 points); (vii) current German President and Chancellor (4 points); (viii) finger tapping (2 points); and (ix) word opposites (2 points). The instrument includes 4 cognitive domains: orientation; memory (registration, recent memory and delayed recall); attention/calculation; and language (semantic memory, comprehension and repetition). The TICS-m was administered according to

published procedures and followed a standardized script.[25] The TICS-m score ranges from of 0 to 50.[24] Normal cognitive function was defined as having a TICS-m score ≥ 31; mild cognitive impairment (MCI) was between 27–31; and a score ≤ 27 indicated probable dementia.[26]

Exposure: Sleep Characteristics

Sleep related characteristics were evaluated in the interview using the Uppsala Sleep Inventory (USI).[27] Two separate 3-category interview questions were asked concerning an individual's difficulties initiating sleep ("Do you have trouble falling asleep?") and difficulties maintaining sleep ("Do you wake up during the night?"). Individuals were considered to have difficulties if they answered "often" or "sometimes" and were compared to participants who answered "almost never" in response to the questions. Participants were grouped into those with difficulties initiating sleep (DIS), those with difficulties maintaining sleep (DMS), or those with difficulties in initiating or maintaining sleep (both DIS and DMS). Daytime sleepiness was assessed by the question, "Do you feel tired or exhausted during the day due to the sleeping problems in the night (often, sometimes, or almost never)." Sleep duration was assessed with the question "How many hours a day do you usually sleep? Please also think of nap habits." Participants were invited to answer in integer hours. Extreme sleep duration categories (short: ≤ 5 h, long: ≥ 9 h) were assessed based on the categories described in previous studies.[16,28]

Covariates Assessment

Sociodemographic Variables

Sociodemographic variables included age and sex.

Behavioral and Cognitive Risk Factors

Baseline information on sociodemographic variables, smoking habits, physical activity level, and alcohol consumption were gathered by trained medical staff during a standardized interview. Study participants provided information about whether they had ever smoked cigarettes regularly (never smoked, current smoker). Alcohol consumption was rated as "daily or almost daily," "once or several times a week," "no or very rarely alcohol." Each participant was questioned regarding his or her leisure time physical activity during the winter and summer. The questionnaire consisted of a 4-level graded scale for leisure time physical activity during summer and winter time (0, < 1, 1 to 2, and > 2 h/week). The winter and summer responses were combined to create one variable of leisure time physical activity level. Participants were classified as active if they regularly participated in sports ≥ 1 h/week in either season. Actual hypertension was defined as blood pressure values ≥ 140/90 mm Hg and/or use of antihypertensive medication. Multi-morbidity was defined as the co-occurrence of > 2 disease conditions based on the Charlson Comorbidity Index.[29]

Psychological Variables

Depressive symptoms were measured by the 15-item German version of the Geriatric Depression scale ([GDS-15] cutoff point > 5 for mild or moderate depression).[30]

Statistical Analysis

Baseline Descriptive Analysis

Study population characteristics were stratified by a cognitive status at baseline (normal versus cognitively impaired (probable dementia and MCI). Participants were classified as having no sleep problems (n = 159), having DIS (n = 52), DMS (n = 211) or both DIS and DMS (n = 318). Cognitive change (TICS-m1-TICS-m2) was defined as the difference between TICS-m score value at baseline and follow-up. Participants who had improved or declined in cognitive score at follow-up were based on a one point change per year (± 3). A negative value represents an improvement in cognitive function and a positive value represents cognitive decline. Bivariate associations of baseline variables with cognitive status were tested using the Kruskal-Wallis test for continuous variables with more than 2 groups. The χ^2 test was used to examine the associations between categorical variables.

Analytic Statistics

Linear regression was carried out to analyze the association between various patterns of poor sleep (main exposure) and cognitive change (continuous outcome). Three distinct patterns of poor sleep (sleep difficulties at night [DIS, DMS, and both], daytime sleepiness, and sleep duration) and the total sum score of all sleep domains were considered in separate models with 2 approaches. The association of poor sleep patterns and cognitive decline was considered in one "crude model" adjusted only for age and sex as well as in a "full model" adjusted additionally for the presence of known potential baseline confounders (age, sex, baseline cognitive score, smoking status, alcohol consumption, physical activity, depressive symptoms, hypertension, and somatic comorbidities[9,10,12,13,15,19–21]). Crude and fully adjusted models were calculated by using the GLM (generalized linear models) procedures. We then examined the interaction of the effect of any of the 3 patterns of poor sleep with baseline cognitive status on cognitive decline, by including the product of both variables in the fully adjusted model. Results are presented as parameter estimates of unstandardized (β) and 95% confidence intervals (CI). All statistical analyses were run in SAS version 9.2 (SAS Institute Inc., Cary, NC). The significance level was set at 0.05. The analysis and description followed the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for observational studies.[31]

RESULTS

Descriptive Analyses

The baseline study population consisted of 740 subjects including 49% (n = 366) men and 51% (n = 374) women. The median age of participants at baseline was 75 years (SD ± 6.18) ranging from 64–94 years. Among all participants, 82.83% (n = 613) had normal cognitive status, 13.51% (n = 100) showed indications of mild cognitive impairment (MCI), and 3.64% (n = 27) of participants had scores indicating probable dementia at baseline. Table 2 summarizes the sociodemographic characteristics and mental health related

Table 2—Population characteristics stratified by baseline cognitive status (n = 740).

Variables	Baseline Cognitive Status		P value
	Normal (n = 613)	Cognitively Impaired (n = 127)	
Age, mean ± SD (74.59 ± 6.18)	74.16 ± 6.16	76.77 ± 5.78	0.15
TICS-m score, mean (95% CI)	37.4 (37.1 to 37.7)	28.7 (28.3 to 29.1)	< 0.0001
Cognitive change score, mean (95% CI)	−1.28 (−2.10 to −0.45)	0.76 (0.42 to 1.10)	< 0.0001
Male	286 (46.66)	80 (62.99)	
Female	327 (53.34)	47 (37.01)	
Alcohol consumption			0.33
Daily or almost daily	175 (28.55)	45 (35.43)	
Once or Several times a week	176 (28.71)	30 (23.62)	
Smoking status			0.27
Current smoker	254 (41.44)	46 (36.22)	
Never smoked	359 (58.56)	81 (63.78)	
Physically inactive	234 (38.17)	64 (50.39)	0.01
Hypertension	444 (72.43)	94 (74.02)	0.71
Multi-morbidities			0.11
No somatic disease condition	69 (11.26)	8 (6.30)	
One condition	173 (28.22)	31 (24.41)	
≥ 2 conditions	371 (60.52)	88 (69.29)	
Sleep problems			0.45
Only DIS	46 (7.50)	6 (4.72)	
Only DMS	179 (29.20)	32 (25.20)	
Both DIS and DMS	260 (42.41)	58 (45.67)	
Daytime sleepiness			0.52
Often/sometimes	208 (33.93)	37 (29.36)	
Almost never	404 (66.01)	90 (70.87)	
Sleep duration, mean ± SD (7.67 ± 1.40)	7.62 ± 1.36	7.94 ± 1.56	0.98
Short sleep hours	39 (6.4)	9 (7.1)	0.60
Long sleep hours	147 (24.0)	43 (33.9)	0.06
Depressive symptoms	6 (0.98)	2 (1.57)	0.55

Values are presented as n (%), mean ± SD, or mean (95% CI). For continuous variables P values are derived by Kruskal-Wallis tests. For categorical variables P values are derived by χ^2 tests. SD, standard deviation; CI, confidence interval; DIS, difficulties in initiating sleep; DMS, difficulties in maintaining sleep.

variables stratified by cognitive status at baseline. Compared to cognitively impaired individuals, subjects with normal cognition were more likely to be female and to be physically active. However, at baseline, no significant differences between the 2 groups were observed in terms of age, lifestyle and cardiovascular risk factors (smoking status, alcohol intake, and hypertension), sleep characteristics (difficulties in maintaining sleep [DMS], difficulties in initiating sleep [DIS], daytime sleepiness [DS], and sleep duration), and depressive symptoms.

As displayed in Table 2, in all 613 study participants with normal cognitive function at baseline, a total of 21% (n = 128) were individuals without sleep problems, whereas a minority (8%, n = 46) reported having DIS, 29% (n = 179) reported DMS, and 42% (n = 260) had both DIS and DMS. Among 127 cognitively impaired subjects, 24% (n = 31) reported no sleep problems, whereas 5% (n = 6) reported having DIS, 25% (n = 32) had DMS, and 46% (n = 58) had both DIS and DMS.

Prospective Analysis

Cognitive Trajectories of the Study Participants

In the total population, the mean (± SD) TICS-m score at baseline and at 3-year follow up was quite similar (baseline = 35.9 ± 4.8 and follow-up = 35.5 ± 5.5). Almost half of the population (43%, n = 317) remained stable in their cognitive status. In 32% of participants (n = 236), a decline in cognitive score was observed, while in another 25% of participants (n = 189), > 3-point improvement in their TICS-m score was observed. Thus explaining the overall stability of TICS-m score in the population over 3 years.

In subjects with normal cognitive function (n = 615), 35% (n = 214) showed a decline after 3-year follow-up in cognitive score, 22% (n = 134) improved, and 43% (n = 267) remained stable in their cognitive score. From baseline to follow-up, the decline in cognitive score (mean, 95% CI) of participants with normal cognition was −1.28 (−2.10 to −0.45). However, the mean of change in score of the TICS-m

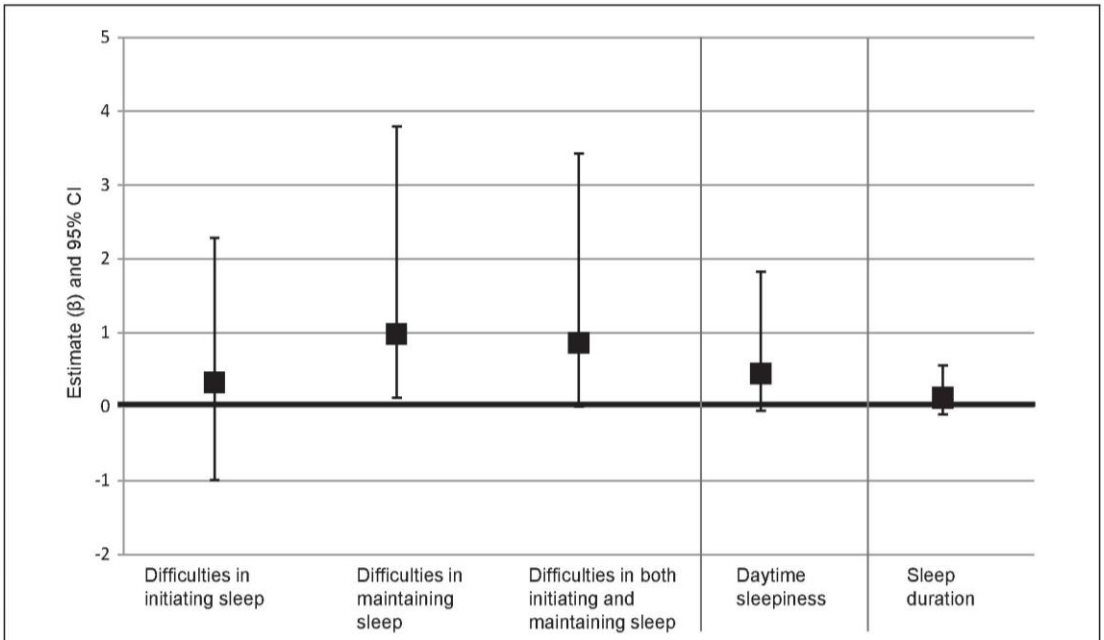

Figure 2—The effect (β-estimate and 95% confidence interval (CI)) of patterns of poor sleep on cognitive decline (n = 740). (Exposure of patterns of poor sleep on cognitive decline were assessed in 3 separate models; model 1: difficulties in initiating sleep, difficulties in maintaining sleep and both; model 2: daytime sleepiness; model 3: sleep duration). The effect of difficulties initiating, or maintaining sleep, or both, were referenced to no sleep difficulties. The effect of daytime sleepiness (often and sometimes) was referenced to almost never daytime sleepiness.

score for individuals with normal cognition remained within the normal range.

Out of 127 cognitively impaired participants, 17% (n = 22) showed a decline in cognitive score while 43% (n = 55) improved in their cognition and 39% (n = 50) remained stable. The mean of change in TICS-m score was 0.76 (0.42 to 1.10). Although the mean of change in score showed an improvement, the change in score remained within the cutoff for cognitive impairment.

Associations between Patterns of Poor Sleep and Cognitive Decline over Three Years

Participants who reported DMS at baseline (n = 211) were more likely to have a decline in their cognitive score after 3 years compared to those who reported no DMS at baseline (β = 0.85, 95% CI = 0.001 to 1.70, P value (P) = 0.049) (crude model adjusted for age, sex, and baseline cognitive score). The association between DMS and cognitive decline was slightly increased when models were additionally adjusted for physical activity, hypertension, smoking status, alcohol consumption, co-morbidities and depressive symptoms (full model, β = 0.88, 95% CI = 0.03 to 1.74, P = 0.04) as depicted in Figure 2.

However, difficulties in initiating sleep (DIS) (n = 52) or combined difficulties in maintaining and initiating sleep (both DIS and DMS) (n = 318) were not significantly associated with cognitive decline (DIS: β = 0.29, 95% CI = −1.01 to 1.59, P = 0.66, both DIS and DMS: β = 0.63, 95% CI = −0.19 to 1.44, P = 0.13), although the direction of association was similar. No other sleep characteristics (DIS, DIS and DMS, DS, or the continuous sleep duration variable) achieved statistically discernable effects. Daytime sleepiness and sleep duration were also assessed as main exposures in crude and full models (Figure 2). In a sensitivity analysis, extreme sleep duration categories (very short or very long sleep hours) were not significantly associated with cognitive decline in the total sample (data not shown).

Stratified Analysis: Associations between Sleep Disturbances and Cognitive Decline Stratified by Cognitive Status at Baseline

Based on the known confounding role of baseline cognitive status as well the significant interaction observed between cognitive decline and sleep disturbances (DMS*baseline cognitive status: P = 0.04), we further stratified our regression analyses according to normal or impaired cognitive status at baseline. DMS was significantly associated with cognitive decline only in participants with normal cognitive status and not in cognitively impaired individuals. In a fully adjusted model, cognitive decline was 1.3-points larger in cognitively normal individuals with DMS compared to individuals with no sleep disturbances (β = 1.26, 95% CI = 0.35 to 2.18, P = 0.007) whereas the effect in cognitively impaired participants with a 0.06-point change in TICS-m score, was not statistically significant in the model (β = 0.06, 95% CI = −2.23 to 2.35, P = 0.96) (Table 3).

Table 3—The effect of sleep-related characteristics (sleep disturbances, daytime sleepiness, and sleep duration) on cognitive decline stratified by baseline cognitive status (n = 740).

	Normal (n = 613)		Cognitively Impaired (n = 127)	
Sleep Characteristics (n)	**β (95% CI)**	**P value**	**β (95% CI)**	**P value**
No sleep problems	0	0	0	0
Only DIS (46/6)	0.58 (−0.78 to 1.94)	0.40	−1.40 (−5.39 to 2.58)	0.49
Only DMS (179/32)	1.26 (0.35 to 2.18)	0.007	0.06 (−2.23 to 2.35)	0.96
Both DIS and DMS (260/58)	0.77 (−0.11 to 1.66)	0.09	0.23 (−1.98 to 2.45)	0.83
No daytime sleepiness, DS	0	0	0	0
Often (64/10)	−1.05 (−2.22 to 0.11)	0.07	−1.82 (−5.29 to 1.65)	0.31
Sometimes (144/27)	0.11 (−0.67 to 0.89)	0.77	0.65 (−2.65 to 1.34)	0.52
Sleep duration, hours	0.006 (−0.23 to 0.24)	0.96	0.45 (−0.05 to 0.96)	0.08
6–8 h (38/9)	0	0	0	0
Short, ≤ 5 h (429/75)	0.66 (−0.68 to 2.01)	0.99	−0.14 (−3.38 to 3.09)	0.93
Long, ≥ 9 h (146/43)	0.37 (−0.38 to 1.13)	0.12	1.86 (0.15 to 3.57)	0.03
Sum of sleep score				
0 (78/19)	0	0	0	0
1 (164/28)	1.19 (0.11 to 2.27)	0.03	2.89 (0.21 to 5.58)	0.03
2 (179/38)	1.14 (0.07 to 2.22)	0.04	1.47 (−1.06 to 3.99)	0.25
3 (155/29)	1.16 (0.03 to 2.30)	0.04	1.06 (−1.73 to 3.84)	0.46
4 (39/13)	0.95 (−0.67 to 2.57)	0.25	3.36 (−0.23 to 6.95)	0.07

Adjusted for age, sex, baseline cognitive score, physical activity, hypertension, smoking status, alcohol consumption, comorbidities, and depressive symptoms. DIS, difficulties in initiating sleep; DMS, difficulties in maintaining sleep; DS, daytime sleepiness.

The impact of DMS was assessed in a sensitivity analysis as a single sleep complaint, adjusted additionally for DIS and other important confounders. As expected, DMS significantly increased the risk for cognitive decline in individuals with normal cognition (β = 0.89, 95% CI = 0.15 to 1.64, P = 0.02) while DIS did not (−0.23, −0.93 to 0.46, 0.52). Also no significantly discernable association was observed between either DMS or DIS and cognitive impairment in the cognitively impaired subjects (DMS: 0.47, −1.51 to 2.46, 0.64, DIS: −0.15, −2.04 to 1.74, 0.88). However, the accumulation of sleep complaints increased the risk for cognitive decline, as displayed in Table 3 in individuals with normal cognition. Here, a suggestive dose-response relationship was observed (1: β = 1.19, 95% CI = 0.11 to 2.27, P = 0.03, 2: 1.14, 0.07 to 2.22, 0.04, 3: 1.16, 0.03 to 2.30, 0.04). On the other hand, as also displayed in Table 3, prolonged sleep duration increased the risk for cognitive decline only in cognitively impaired participants (1.86, 0.15 to 3.57, 0.03) and not in subjects with normal cognition (0.37, −0.38 to 1.13, 0.12). No significant association between short sleep duration and cognitive decline was observed.

DISCUSSION

Insomnia comprises an impaired sleep pattern with difficulties in initiating sleep (DIS), maintaining sleep (DMS) or non-restorative sleep accompanies by significantly impaired daytime functioning.[32] Sleep problems are frequently reported in the elderly[33] and this is confirmed in the present study with only approximately 25% of participants reporting no sleep problems. To the best of our knowledge, here we present the first study in an older, community-based population that reports a significant association between DMS and cognitive decline in men and women with normal cognitive status over a 3 year of follow-up.

Even the adjustment for confounding risk factors (physical activity, hypertension, smoking status, alcohol intake and multi-morbidity) did not alter the strength nor the significance of the prospective association between DMS and cognitive decline in these subjects. This also holds true for depression, which is an established risk factor for cognitive decline[8] and is a key pattern of impaired sleep,[34] making it unlikely that the relationship between sleep and cognitive decline could be independent of depressive symptoms. However, the association between sleep disturbances and cognitive decline remained significant even after further adjustment for depressive symptoms. Hence, DMS appears to be a robust and independent predictor of cognitive decline particularly in subjects with normal cognitive function.

A positive association of DMS on cognitive decline has already been demonstrated in a few longitudinal studies, however, this evidence is mostly limited by sex-specific samples. The population-based MrOS study of 2,822 elderly men,[7] the male population in the Established Populations for Epidemiologic Studies of the Elderly (EPESE) study (n = 6,444, age 60 years and above),[8] and the elderly female population of the Survey on Elders' Health (ESA) study with 1,664 subjects (age range of 65 to 96 years)[28] showed an association between DMS and cognitive decline. In contrast, no association was observed between sleep disturbances and incident cognitive impairment in a female cohort of the Nurses' Health Study cohort (n = 1,844, age 70 to 81 years).[16] Thus, the present investigation confirms and extends these previous findings for both sexes in which DMS is associated with cognitive decline.

Furthermore, the previous studies of the EPESE study, the Maastricht Ageing Study (MAAS) and the Nurses' Health Study cohort, have combined DIS and DMS as part of insomnia-like features or subjective sleep complaints and did not assess DIS and DMS separately.[8,16,19] In this present study, we have shown that only DMS, and not DIS, nor both DIS and DMS, was associated with cognitive decline. DMS was still the only significant predictor of cognitive decline, even when assessed as a single sleep complaint in a model adjusted for DIS.

DMS has no measureable effect on cognitive decline in participants who were already compromised by lower cognitive functioning at baseline. No other study has comprehensively assessed the modifying role of the baseline cognitive status in the association of DMS and cognitive decline. The null effect in the cognitively impaired participants may be due to a "floor effect" resulting from little change in TICS-m score at baseline to follow-up because cognitively impaired subjects had already reached a lower cognitive function whereas individuals with normal cognition had a larger room for improvement/decline due to a wider range of TICS-m score in the normal cognitive status category. Secondly, we cannot exclude the possibility that cognitively impaired participants were not able to correctly answer the TICS-m questions.

Difficulties in initiating sleep (DIS) and daytime sleepiness (DS) were not associated with diminished cognition. As DIS has been associated in earlier studies with cognitive decline, it is most likely that our nonsignificant finding is due to the low number of subjects who reported DIS. The same holds true for daytime sleepiness (DS) which was recently demonstrated to be independently associated with cognitive decline in men and women of the French Three-City Study (n = 4,894 participants).[10] Additionally, in the Honolulu-Asia Aging study (n = 2,346) of men, daytime sleepiness (DS) was highlighted to be the only measurement that was reliably associated with cognitive decline over a 3-year follow-up.[9] On the contrary, in a British study of men and women (n = 2,041, aged 65 years and older), daytime napping was associated with lower risk of cognitive decline.[11] The present investigation contributes novel data to conflicting evidence published to date. A meta-analytic research strategy which could clarify the impact of DS in cognitive decline is urgently needed.

The third sleep feature assessed in this present investigation was sleep duration (in hours) that was neither associated with TICS-m score nor with cognitive decline; a result which is supported by a previous study.[16] In study participants with probable cognitive impairment at baseline, individuals who reported extremely long sleeping duration (≥ 9 h) had a significantly increased risk of continued cognitive decline. This was not observed with short sleep duration (≤ 5 h). However, it should be mentioned that both short[11] and prolonged sleep hours[12,13,15,20,28] have been shown to be associated with cognitive decline in other prospective analyses. Interestingly, long sleeping, as assessed by self-report and not from objective actigraph measurement, was significantly associated with cognitive decline in a cross-sectional male population study (n = 3,132, age 76.4 ± 5.6 years).[4] Thus, the current finding highlights the deleterious effect of prolonged sleep duration in cognitively impaired elderly men and women.

Potential Mechanisms

The present study was not designed to elucidate the psychobiological mechanism to explain the link between DMS and cognitive decline. However, insomnia is a sleep disorder associated with both cognitive-emotional and physiological hyperarousal. Dysregulated hypothalamic-pituitary-adrenal (HPA) axis[35] accompanied by hypersecretion of cortisol levels[36] and proinflammatory cytokines[37] were associated with increased risk for cognitive decline. Chronic exposure to cortisol[38,39] and blunted diurnal cortisol pattern[39] as well as central cholinergic dysfunction[40] with advancing age, may contribute to neurotoxicity effects on the hippocampus, which can affect memory performance and lead to cognitive impairment. It has been shown that the sleep maintenance zone is important for cognitive function[41] which occurs at the peak of REM when the body temperature is lowest.[42] Disruption of REM sleep has long been known to be associated with cognitive decline and a recent population-based study further identified specific stages within REM to be associated with cognitive decline in older men.[43] Our current analyses extend these findings in both sexes with evidence that DMS may be disrupting this essential phase of sleep that leads to memory failure. Future studies are warranted to assess disrupted sleep patterns with diurnal cortisol patterns to complete the link between HPA axis dysregulation, disrupted sleep patterns and cognitive decline.

Besides the detrimental effects of DMS on cognitive decline in elderly individuals with normal cognition, the findings that prolonged sleep hours have a negative impact on cognition appears somewhat counterintuitive. However, the fact that this was only observed in cognitively impaired participants may reflect a clinical symptom of deterioration with old age. It is not unlikely that this finding reflects a syndrome of vital exhaustion and general decline in somatic abilities and thus could also be a sign of a disease condition such as depression, frailty, or neurological disorders.

Study Strengths and Limitations

The study has several strengths and limitations. Among the strengths of the study is the extensive assessment of health risk factors in a large sample of elderly participants, enabling us to perform a comprehensive adjustment for potential confounders. The current study also has the advantage of using the TICS-m measurement that is sensitive for detecting early cognitive impairment and useful in large study settings[24] with a good test-retest reliability.[44] However, one needs to acknowledge that the TICS-m score is a screening instrument which does not substitute for a clinical diagnostic procedure but has significant probability to distinguish between normal and impaired cognition. The distribution of the TICS-m has been shown to have a more symmetric and normal-shaped distribution than the Mini-Mental State Examination (MMSE), suggesting that TICS-m is less subject to the ceiling effects.[24] Furthermore, both cognitively healthy and cognitively impaired individuals were phenotyped at baseline. The study participants were healthy enough to conduct a telephone interview and highest quality assurance was incorporated into the study to assure that each participant was independently answering each question.

A potential limitation of this study was the relatively short follow-up period of 3 years, which may have been too short to capture discrete levels of cognitive decline. Nonetheless, 3 years in old age may contribute to substantial changes in general health. Another limitation of this study was the assessment of sleep problems without a fixed time-frame and by self-report. However, only one prospective study assessed both objective and subjective sleep measurements and found no significant difference between both measurements.[7] Furthermore, we did not assess sleep apnea, which may be associated with limited oxygen supply that could have consequences on cognitive functioning.

CONCLUSION

This study presents evidence for the prospective association between longer nighttime wakefulness and cognitive decline over 3 years of follow-up even after adjustment for important confounders. In particular, individuals with normal cognition at baseline were at risk if they experienced difficulties maintaining sleep. These new findings may contribute to the development of appropriate preventive approaches in the identification of impaired sleep quality to avoid cognitive decline in elderly individuals with normal cognitive function. DMS in older adults could be an early warning sign of increased risk of cognitive decline and should receive particular attention from clinicians. Thus, it is important to include sleep quality assessment in health screening for older people. Identification of risk factors, combined with early diagnosis and intervention, is critically important to prevent individuals from cognitive decline that leads on a trajectory toward dementia. In this regard, disrupted sleep may be particularly detrimental for optimal maintenance of cognitive function. Thus, it is important to preserve sleep quality, particularly in the sleep maintenance zone.

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Author contributions: Drs. Emeny and Ladwig designed the current analysis and proofread the manuscript. Drs. Emeny and Ladwig and Ms. Johar proofread the manuscript. Dr. Emeny and Ms. Johar advised on statistical analysis. Ms. Johar and Ms. Kawan managed the literature searches, statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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10. Publications

1. Johar H, Emeny RT, Bidlingmaier M, Reincke M, Thorand B, Peters A, Heier M, Ladwig KH. **Blunted diurnal cortisol pattern is associated with frailty: a cross-sectional study of 745 participants aged 65 to 90 years.** J Clin Endocrinol Metab. 2014 Mar;99(3):E464-8. doi: 10.1210/jc.2013-3079.

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3. Johar H*, Kawan R*, Emeny RT, Ladwig KH.
   Impaired Sleep Predicts Cognitive Decline in Old People: Findings from the Prospective KORA Age Study.
   Sleep. 2015 Aug 31. pii: sp-00785-14.
   * These authors shared the first authorship

Further publications

1. H. Johar, R. T. Emeny, M. Bidlingmaier, J. Kruse, and K.H. Ladwig. **Sex-related differences in the association of salivary cortisol levels and type 2 diabetes. Findings from the cross-sectional population based KORA-Age Study.** Submitted to **P**sychoneuroendocrinology, 2015 Oct.
2. H. Johar, R. T. Emeny, M. Bidlingmaier, KH Ladwig. **Cortisol, social network and loneliness in the elderly: findings from the cross-sectional KORA Age study.** In preparation
3. H. Johar, M. Bidlingmaier, W. Koenig, B. Thorand, K.H. Ladwig. **Chronic stress exposure in the elderly may compromise the non-inflammatory properties of glucocorticoids.** In preparation.

11. Affidavit

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Medical

Affidavit

I, Hamimatunnisa Johar, hereby declare, that the submitted thesis entitled:

Cortisol secretion patterns in the elderly: in the perspectives of frailty and cognitive function and sleep disturbances as risk factors of cognitive decline

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

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