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Do Physical Activity, Diet and Sex Modify the Association Between Neurotrophin Single Nucleotide Polymorphisms and Insomnia?

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DO PHYSICAL ACTIVITY, DIET AND SEX MODIFY THE ASSOCIATION BETWEEN NEUROTROPHIN SINGLE NUCLEOTIDE POLYMORPHISMS AND INSOMNIA?

by

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ABSTRACT

DO PHYSICAL ACTIVITY, DIET AND SEX MODIFY THE ASSOCIATION BETWEEN NEUROTROPHIN SINGLE NUCLEOTIDE POLYMORPHISMS AND INSOMNIA?

by

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Utah State University, 2024

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The prevalence of insomnia in late life is influenced by a wide array of genetic and lifestyle factors, some of which exhibit sex-dependent effects. This study examined the main effects of selected single nucleotide polymorphisms or SNPs related to the neurotrophin, brain-derived neurotrophic factor (BDNF), or its receptors in relation to lifestyle factors of physical activity and diet, and their interactions on the risk for sleep disturbance in older males and females. This thesis analyzed the initial wave of data from the Cache County Study on Memory and Aging (CCSMA), a longitudinal, population study, where 5,092 participants aged 65 years and older, residing in Cache County, Utah, were assessed through various measures. The results revealed that SNPs for BDNF or its receptors showed no significant association to sleep disturbance in both males and females. Males experienced an 18% reduced odds of sleep disturbance with increasing levels of physical activity (p = .023), whereas unexpectedly, greater adherence to the Mediterranean diet was associated with an 8% higher odds of reporting sleep disturbance.

For females, no significant associations were found between the SNPs, lifestyle factors, and sleep disturbance. However, a trend for an interaction between one SNP by physical activity emerged among females: those with the Val66Met minor allele who engaged in sedentary-to-light physical activity exhibited a 45% higher risk of sleep disturbance compared to those with moderate-to-vigorous physical activity. While there were few main effects of SNPs related to BDNF or its receptors and lifestyle factors in relation to sleep disturbance, trends suggest an intricate interplay of genetic and lifestyle factors with sex-dependent variations in their impact. Future studies may wish to further explore sex-dependent associations between genes and lifestyle factors in improving sleep disturbance in older adults.

(90 Pages)

PUBLIC ABSTRACT

DO PHYSICAL ACTIVITY, DIET AND SEX MODIFY THE ASSOCIATION BETWEEN NEUROTROPHIN SINGLE NUCLEOTIDE POLYMORPHISMS AND INSOMNIA?

Hector Leonardo Gonzalez

Sleep disturbance is common in older adults at prevalence rates ranging between 30 - 50% in the United States. Neurotrophins such as brain-derived neurotrophic factor (BDNF), play a role in sleep (Bachmann et al., 2012) as do lifestyle factors such as physical activity (Dolezal et al., 2017) and diet. This study examined the associations of selected single nucleotide polymorphisms or SNPs related to BDNF or its receptors and lifestyle factors of physical activity and diet, as well as their interactions on the risk for sleep disturbance in older adult males and females. This thesis examined existing data from the Cache County Study on Memory and Aging (CCSMA), a longitudinal, population study of 5,092 individuals aged 65 years and older residing in Cache County, Utah. The results suggest that SNPs related to BDNF or its receptors were not related to sleep problems in either males or females. In males, increased physical activity reduced the likelihood of experiencing sleep disturbances, while unexpectedly, greater adherence to the Mediterranean diet slightly increased the likelihood of reporting sleep problems. In females, the SNPs and lifestyle choices did not appear to have any relationship with sleep disturbance. However, an interaction between the BDNF gene Val66Met and physical activity showed a trend. Specifically, females with the minor and less common allele who reported sedentary-to-light physical activity exhibited a 45% increase in risk of sleep

disturbance compared to those who reported moderate-to-vigorous physical activity. Overall, this study suggests that SNPs related to BDNF or its receptors had no significant association with sleep disturbance, but that some effects may be specific to males or females. Increasing physical activity may be beneficial for males with sleep disturbance as well as for females, but for the latter, only for a certain BDNF genetic profile. Future studies may wish to further explore sex-dependent associations between genes and lifestyle factors in improving sleep disturbance in older adults.

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Hector Gonzalez

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Chapter I

Introduction

Insomnia is a major health problem that affects nearly 60 million Americans each year (Colten et al., 2006). The adverse effects of the high prevalence of insomnia or some variant of sleep disturbance in the U.S. contribute to poor performance in school (Dewald et al., 2010) and the labor market (Kessler et al., 2011). It is well established that insufficient sleep has a negative effect on physical (e.g., hypertension, obesity, diabetes; Paruthi et al., 2016) and mental health (e.g., depression, anxiety, suicidality; Reid et al., 2006). Compared to the general population, older adults experience disproportionately high prevalence rates of insomnia, ranging between 30 - 50% (Ohayon, 2002; Peng et al., 2021; Foley et al., 2004; and Li et al., 2018). Researchers suggest that sleep deprivation may promote cognitive changes and facilitate the development of neurodegenerative diseases and cognitive decline (McEwen, 2006; Yaffe et al., 2014). Recent studies have proposed that the impact of sleep on well-being and cognition may be further enhanced in old age (Kocevska et al., 2021). Given the significance of sleep to health and well-being, further research is necessary to identify specific risk factors for sleep disturbance.

To understand the factors that distinguish individuals with normal sleep from those experiencing sleep disturbances, it is beneficial to consider both biological and lifestyle factors. Neurotrophins such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), play a critical role in neuroplasticity (Duman et al., 2000; Pittenger & Duman, 2008) as well as the regulation of all stages of sleep, at least with respect to BDNF (Faraguna et al., 2008; Deuschle et al., 2018; Guindalini et al., 2014). Individuals with insomnia have shown lower serum BDNF levels compared to

individuals without insomnia (Giese et al., 2013). Moreover, single nucleotide polymorphisms (SNPs) related to BDNF signaling have also been associated with sleep regulation, as demonstrated by electroencephalographic (EEG) activity (Bachmann et al., 2012; Guindalini et al., 2014). No studies have examined NGF in relation to sleep or sleep disturbances.

Lifestyle factors such as physical activity and diet affect sleep quality and duration. Physical activity ranging from low to high intensity has shown an association with enhanced sleep efficiency and duration (Dolezal et al., 2017; Du et al., 2015). Independent of sleep quality, physical activity has also been reported to increase BDNF levels (Griffin et al., 2011; Berchtold et al., 2005). Similarly, nutrient-rich diets have been shown to improve sleep duration (Ikonte et al., 2019; Grandner et al., 2013). BDNF levels may also be contingent on macronutrient intake such as the intake of fats, proteins, and carbohydrates (Molteni et al., 2002), which have been associated with sleep quality (Sanchez-Villegas et al., 2011).

Previous research has identified a moderating effect of diet and physical exercise on the relationship between BDNF levels and sleep. In an animal study, a Western diet high in fat (25% total fat) was associated with decreased BDNF levels in the hippocampus and poor sleep (Alzoubi et al., 2013). In a review by Tan et al. (2020), physical activity was correlated with increased BDNF signaling and sleep. However, potential interactions between lifestyle factors (i.e., diet and physical activity) and BDNF-related genes or SNPs and risk for sleep disturbance have been largely unexamined.

This study addressed a gap in the current research literature by examining the associations between the outcome of sleep disturbance and SNPs for BDNF or its receptors along with lifestyle factors of physical activity and diet, in a large, population-based sample of older adults. Specifically, I examined whether SNPs for BDNF and its receptors moderated the relationship between physical activity/diet and sleep disturbance. Sex differences, which have not previously been examined, were explored. The present study added to the existing research by expanding knowledge of the role of lifestyle factors in combination with specific SNPs coding for BDNF and its receptors on the risk for sleep disturbances.

Chapter II

Literature Review

Insomnia is considered an umbrella term for all types of sleep disturbances. The American Academy of Sleep Medicine defines it as difficulty with sleep initiation, duration, consolidation, or quality (American Academy of Sleep Medicine [AASM], 2005). The prevalence rates of insomnia increase with age, with rates of 6.1% in children, 9.1% in adolescence, 11.3% in young adults, and 20.2% in older adults (Kocevska et al., 2021). Several epidemiological studies have also reported prevalence rates of insomnia in older adults from any cause to range between 30 - 50% (Ohayon, 2002; Peng et al., 2021; Foley et al., 2004; and Li et al., 2018). Among otherwise healthy older adults, more common causes of sleep disturbances include obstructive sleep apnea affecting between 13 and 32% (Carlisle et al., 2014), restless leg syndrome affecting between 10 to 35% (Milligan & Chesson, 2002), major depressive disorder affecting between 62-88% (Yates et al., 2004), and anxiety disorders affecting between 14–17% (Canuto et al., 2018; Norton et al., 2012; Miloyan & Pachana, 2015). Sex differences in sleep duration and quality have also been reported in older adults, with one study suggesting that women report worse sleep quality, compared to men (Bixler et al., 2002). However, other studies have suggested that women are at higher risk for insomnia (Zhang & Wing, 2006) and restless legs syndrome (Berger et al., 2004), whereas men reportedly are at higher risk for obstructive sleep apnea (Jordan & McEvoy, 2003).

Sleep disturbance may impact overall health and level of functioning with far-ranging effects on cardiovascular health, metabolic health, mental health, cancer, pain, overall mortality, and the immune system (Paruthi et al., 2016). Sleep disturbance has

also shown an association with an increased risk of dementia and cognitive decline (Yaffe et al., 2014). Studies suggest that non-rapid eye movement sleep (NREM; stages 1 – 4), characterized by slow-wave activity (SWA), is associated with restorative physiological processes (Knyazev, 2012), while rapid eye movement (REM; stage 5) sleep is associated with the consolidation of declarative (Feld & Diekelmann, 2015) and emotional memories (Glosemeyer et al., 2020). The restorative nature of SWA sleep has been associated with sleep quality and is regarded as the best-established correlate of sleep duration and intensity (delta power; Faraguna et al., 2008).

Brain Derived Neurotrophic Factor (BDNF) and Sleep

Biological and lifestyle factors have been associated with sleep and sleep disturbance. Neurotrophins such as brain derived neurotrophic factor (BDNF), play a critical role in neuroplasticity, neurogenesis, and neuronal health and survival (Duman et al., 2000). BDNF has also been implicated in NREM and REM sleep. In a study by Kushikata et al. (1999), 24 male Sprague-Dawley rats and 25 male New Zealand White rabbits were injected intraventricularly with varying doses of BDNF (10 ng, 50 ng or 250 ng) and sleep patterns were monitored via surgically implanted electroencephalographic (EEG) electrodes. Moderate (50 ng) and high (250 ng) doses of BDNF increased the amount of time spent in NREM sleep in rats, but in rabbits, only the highest dose increased the amount of time spent in NREM and REM sleep (Kushikata et al., 1999). In another animal study (Faraguna et al., 2008), researchers examined the relationship between BDNF and SWA sleep on EEG. In this study, rats received one of three cortical microinjections: BDNF, K252a [a blocker of the BDNF receptor tyrosine kinase B (TrkB)], or a control “vehicle” substance. BDNF injections resulted in a significant

increase of SWA sleep, whereas injection of the BDNF TrkB receptor blocker produced a blunted SWA sleep response (Faraguna et al., 2008).

Another study examined “knockout” mice lacking the TrkB receptors (n = 10) and wild-type mice (controls; n =9). Sleep was measured using polysomnographic recordings that employed both EEG and Electromyography (EMG) cables. The TrkB knockout group experienced increased REM sleep and REM bout duration, as well as reduced REM sleep latency and abnormal REM sleep regulation (Watson et al., 2015). These animal studies support the involvement of BDNF and its receptor, TrkB, in sleep regulation.

Human studies have also been conducted, examining the association between BDNF levels and sleep stages. Deuschle et al. (2018) examined middle-aged adults (mean =47.2 years of age, sd = 11.4 years) with sleep disorders: primary insomnia (n = 35), restless leg syndrome (n = 31), idiopathic hypersomnia (n = 17), and narcolepsy (n = 10) or healthy controls (n = 37). Polysomnography measured sleep integrity and brain EEG to characterize the duration of sleep stages for two consecutive nights. Assays for BDNF levels were obtained from blood. Low serum BDNF levels significantly predicted low REM sleep percentage in controls and those with a sleep disorder (Deuschle et al., 2018). Another study analyzed group differences in serum BDNF levels between sleep-disturbed participants (n = 26) and sleep-healthy participants (n = 24). Researchers found that the sleep-healthy group had significantly higher levels of BDNF than the sleep-disturbed group. Additionally, self-report of sleep quality was associated with BDNF levels with those reporting good sleep having higher serum BDNF levels compared to those reporting a sleep disturbance (Giese et al., 2013). Finally, in a study of individuals

with and without insomnia, individuals who endorsed specific forms of insomnia (self-reported difficulties in initiating/maintaining sleep, early awakening, or daytime exhaustion) were divided into two groups: one with short sleep duration (SSD; sleep time < 6 hours, n = 30), and a second with normal sleep duration (NSD; sleep time ≥ 6 hours, n = 27). Compared to the NSD group, individuals in the SSD group had lower serum BDNF levels (Fan et al., 2019). Altogether, animal and human studies provide evidence for the relationship between BDNF and sleep duration and quality, with specific associations noted with the duration of slow wave and REM sleep.

Genetic factors related to BDNF have also been implicated in sleep quality. Single-nucleotide polymorphisms (SNPs) are the simplest forms of DNA that determine the genetic variation between individuals and serve as biomarkers which may be associated with various diseases or conditions (Shastry, 2009). The BDNF gene, rs6265 (BDNF Val66Met), represents a variation in which methionine (Met) is substituted for valine (Val) at codon 66 in the pro-BDNF protein (Park et al., 2017). A study by Bachmann et al. (2012) examined the effects of BDNF Val66MET on sleep regulation, comparing Val/Met (n = 11) and Val/Val (n = 11) genotype groups. Sleep was studied over a 4-day period, with EEG recordings taken before and after 40 hours of sleep deprivation. The Val/Val genotype was linked to increased SWA in NREM sleep delta, theta and alpha activity following the 40-hour sleep deprivation when compared to the Val/Met genotype (Bachmann et al., 2012). This finding suggests that the Val/Val genotype elicited greater sleep pressure (need for sleep), and increased SWA compared to those with the Val/Met genotype. Furthermore, those with the Val/Val genotype benefitted in experiencing an average of 20 more minutes in deep sleep (normal time in

deep sleep is around 1 to 2 hours) than those with the Val/Met genotype (Bachmann et al., 2012). A similar but larger study conducted by Guindalini et al. (2014) found that carriers of the Met allele exhibited decreased theta and alpha activity during NREM sleep when compared to Val/Val carriers. While this pattern did not significantly predict sleep duration, it did suggest that BDNF genotypes are associated with observed EEG activity of the brain during sleep. This study further corroborates the proposition that the BDNF Val66MET gene plays a role in SWA sleep. To our knowledge, no other genes coding for BDNF and its receptors (TrkB or NGF), or other neurotrophins have been examined in human studies.

Lifestyle Factors, BDNF and Sleep

Lifestyle factors such as physical activity and diet have been shown to affect levels of BDNF and sleep quality. Ferris et al. (2007) explored the effects of exercise intensity on serum BDNF levels in 15 young adults (mean age = 25.4 years, sd = 1.0). Participants engaged in one of three intensities of exercise on a cycle ergometer on three separate days. When compared to the pre-exercise baseline, researchers found a significant increase of serum BDNF levels post-exercise (Ferris et al., 2007). Similarly, another study found that both acute and chronic exercise increased concentrations of BDNF in serum, which interestingly was also associated with improved performance on measures of face–name matching and Stroop Word–Color Interference (Griffin et al., 2011). Moreover, researchers have found that increased BDNF levels gained through exercise remain for several days after the activity and could be regained by reinitiating regular exercise after two weeks of inactivity (Berchtold et al., 2005).

Aerobic exercises reportedly have been associated with improved sleep quality (Wang & Youngstedt, 2014), though studies have also reported similar associations regardless of the mode and intensity of activity in middle-aged and older adults (Dolezal et al., 2017). In a comprehensive review (Dolezal et al., 2017), researchers reviewed the relationship between exercise and sleep quality in thirty-four studies. While these studies consisted of participants from a wide range of demographics and physical activity pertaining to age, health status, and type of exercise intervention, the most robust findings came from samples of middle-aged and older adults, where improved sleep efficiency and duration were reported irrespective of exercise mode or intensity (e.g., moderate to intense aerobic exercises, resistance training, or mind-body exercises) (Dolezal et al., 2017). In another systematic review and meta-analysis, researchers analyzed five randomized controlled trials that examined Tai Chi as the main intervention for improving sleep quality in older adults (Du et al., 2015). Tai Chi is a traditional Chinese form of aerobic exercise that is self-paced and is characterized by slow, controlled movement and deep breathing (Taylor-Piliae et al., 2004). All studies employed the Pittsburgh Sleep Quality Index (PSQI), a self-report measure that assesses seven sleep characteristics: sleep disturbance, sleep latency, sleep quality, subjective sleep duration, use of sleep medication, daytime dysfunction, and habitual sleep efficiency. In the meta-analysis, Tai Chi exercises had a large beneficial effect on PSQI global scores and a moderate effect on extending sleep duration and alleviating sleep disturbances (Du et al., 2015).

Studies have also found that diet can impact the regulation of BDNF levels and sleep. Molteni et al. (2002) studied the association between neurotrophins and diets rich

in saturated fat and refined sugar (high-fat sugar or HFS diet) in female rats. Reportedly, two months on an HFS diet resulted in reduced levels of BDNF in the hippocampus and poorer spatial learning performance (Molteni et al., 2002). Another study examining the effects of macronutrients on BDNF in mice found an interaction between high carbohydrate diets and Apolipoprotein E (APOE), a gene with alleles that have been well-documented as a risk factor for Alzheimer's disease (Saunders et al., 1993). Specifically, mice with the APOE ε4 allele on a high carbohydrate diet showed reduced BDNF levels and BDNF-TrkB activity in the hippocampus (Maioli et al., 2012). In contrast, healthy diets have shown the opposite effect on BDNF levels. Multiple studies have shown that diets rich in omega-3 fatty acids upregulate BDNF levels in rats (Tyagi et al., 2013; Wu et al., 2004) and flavonoid-rich foods including blueberries, green tea, or ginkgo biloba, for example, have been associated with increases in hippocampal BDNF levels and enhancements in spatial memory in 18-month-old rats (Rendeiro et al., 2013). Flavonoids are phytonutrients (plant chemicals) and have been characterized as having strong antioxidant and anti-inflammatory properties (Kicinska & Jarmuszkiewicz, 2020). Flavonoid-rich diets have been implicated in improving cognitive function in children (Barfoot et al., 2019), young adults (Whyte et al., 2019), and older adults (Bensalem et al., 2019). Additionally, flavonoids reportedly have sedative properties that may improve sleep quality (Godos et al., 2020; Fernandez et al., 2003; Jiang et al., 2007). The Mediterranean-style diet is one that is rich in omega-3 fatty acids and flavonoids (Huhn et al., 2015). In a study of older adults, Sanchez-Villegas et al. (2011) found higher plasma BDNF levels among participants assigned to one of three diet groups: a control (low-fat) diet, a Mediterranean Diet with virgin olive oil (MeDiet + VOO), or with nuts (MeDiet +

Nuts). The researchers reported a 78% reduction in risk of low plasma BDNF levels in the group assigned to the MeDiet + Nuts group (Sanchez-Villegas et al., 2011). These studies suggest that diets rich in nutrients and specific macronutrients play an important role in regulating BDNF levels.

Several studies have reported a relationship between diet and sleep quality (Ikonte et al., 2019; Grandner et al., 2013). Ikonte et al. (2019) examined the relationship between short sleep duration (<7 hours) and the consumption of micronutrients. Micronutrients are defined as compounds that the body consumes in small quantities (e.g., vitamins, minerals, etc.); in contrast, macronutrients are considered the larger quantity compounds that the body requires such as carbohydrates, proteins, and fats (Savarino et al., 2021). A total of 26,287 adults, aged 19 and older were studied in the National Health and Nutrition Examination Survey (NHANES, 2007–2008) study. Sleep was assessed from a single item, "How much sleep do you usually get at night on weekdays or workdays?" Nutritional intake was examined using "Food Only" or "Food Plus Supplement" (Food + Spp) sources. Regardless of the nutrient source, a greater percentage of individuals who reported a short sleep duration were found to have consumed inadequate amounts of copper, folate, iron, magnesium, riboflavin, zinc, and vitamins A, C, and K. Among a subgroup of middle-aged to older adults (age 51-99 years), researchers found an association between inadequate intake of vitamins A, C, D, E, and zinc and short sleep duration (Ikonte et al., 2019). These findings suggest that diets low in micronutrients are associated with poor sleep. In a separate publication from the NHANES study (Grandner et al., 2013), participants were categorized according to sleep duration into the following groups: very short (<5 hours per night), short (5–6 hours

per night), normal (7–8 hours per night), and long (≥9 hours per night). Compared to the normal sleep duration group, those in the very short (<5 hours) group reported a lower intake of protein, carbohydrates, sugars, dietary fiber, and overall fat (Grandner et al., 2013). Similar findings on macronutrients have been reported by Lindseth et al. (2013), who also reported that diets high in protein were associated with fewer wake episodes, and diets high in carbohydrates were associated with shorter sleep latency.

In a cross-sectional study, researchers were interested in examining the benefits of the Dietary Approaches to Stop Hypertension (DASH) diet on sleep patterns among 1,922 men (mean age = 48.5 years) and 2,019 women (mean age = 50.1 years); Liang et al., 2020). Adherence to the DASH diet (rich in nutrients and low in sodium and fats) was associated with better sleep duration and quality (Liang et al., 2020). Furthermore, a recent review examining the impact of diet on sleep quality and duration in older adults (Gupta et al., 2021) found that adherence to the Mediterranean diet was also associated with sleep quality and quantity. Thus, the research conducted to date suggests that nutrient-rich diets that are low in saturated fats and processed meats, and higher levels of physical activity are associated with sleep duration and quality, as well as the neurotrophin, BDNF.

Summary and Research Questions

In summary, several animal and human studies have established a relationship between BDNF levels and sleep, with higher levels being associated with better sleep quality (Fan et al., 2019), elevated SWA sleep (Guindalini et al., 2014), and increased sleep duration (Fan et al., 2019). While only one SNP, rs6265 (BDNF Val66Met), has been studied with respect to sleep regulation (Bachmann et al., 2012; Guindalini et al.,

2014), other neurotrophin SNPs remain to be examined. Lifestyle factors such as physical activity (Ferris et al., 2007; Griffin et al., 2011) and nutrient-rich diets (Sanchez-Villegas et al., 2011) have also been associated with peripheral BDNF levels. Moreover, physical activity (Dolezal et al., 2017; Du et al., 2015) and diet (Ikonte et al., 2019; Grandner et al., 2013) have also been associated with sleep duration or quality. Largely unexplored, however, are the potential interacting relationships between lifestyle factors and genes/SNPs for neurotrophins or their receptors and sleep or sleep disturbances. Moreover, sex differences have remained largely unexplored in these associations. The purpose of this study was to examine the associations between selected SNPs for BDNF, and its receptors and lifestyle factors of physical activity and diet and their interactions on the risk for sleep disturbance in older adult males and females. The project used extant data from a large, population-based study and addressed the following research questions:

1. Are SNPs that code for BDNF and its receptors associated with the occurrence of sleep disturbance in either males or females?
2. Are lifestyle factors of physical activity, and diet associated with sleep disturbance in either males or females?
3. Are there interactions between SNPs for BDNF and its receptors and lifestyle factors in their associations with sleep disturbance in either males or females?

Methods

Participants

The current study used extant data from the Cache County Study on Memory in Aging (CCSMA), a population-based, longitudinal study of the incidence and prevalence of Alzheimer's disease and related disorders. The Cache County Study's design and procedures have been described in prior publications (see Miech et al., 2002; Breitner et al., 1999). Briefly, the CCSMA began in 1995 in Cache County, Utah, enrolling 5,092 (or 90%) of the county's permanent residents aged 65 years and older. Participants were followed for three subsequent triennial waves involving a multi-staged dementia screening and assessment protocol which was approved by the Institutional Review Boards of Utah State University, Johns Hopkins University, Duke University, and the University of Washington.

Procedures

At baseline (Wave 1), participants were screened with an adaptation (Tschanz et al., 2002) of the 100-point modified Mini-Mental State Exam (3MS) (Teng & Chui, 1987) and completed a risk factor questionnaire regarding their occupational, education, and physical and mental health histories as well as family history of cognitive disorders, and a medication inventory. Participants also provided a buccal sample for extraction of DNA and genotyping. If participants failed to complete the 3MS, scored below 61 or less than 15 points on the orientation section, or were judged as unreliable by the interviewer, a knowledgeable informant was asked to complete the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) (Jorm & Jacomb, 1989). Further dementia screening proceeded for those participants with a 3MS score less than 87 or IQCODE

greater than 3.27 with a telephone interview with a knowledgeable informant using the Dementia Questionnaire (DQ). For participants whose DQ interview was rated as indicative of significant cognitive impairment or possible dementia, the participant underwent a clinical assessment (CA). The CA consisted of a clinical and health interview with an informant and a neurological, neuropsychological, and physical examination of the participant. A study physician, neuropsychologist, and members of the assessment team reviewed the results of the CA to assign preliminary diagnoses of dementia or a cognitive disorder if present. Persons with working diagnoses of dementia or its prodrome were invited to complete neuroimaging, laboratory studies, an examination by a physician and an 18-month follow-up CA. The final determination of dementia and its underlying cause was made by the study's multidisciplinary panel of dementia experts (Breitner et al., 1999), according to criteria from the Diagnostic Statistical Manual-III-Revised (DSM-III-R). Various causes of dementia were determined using standard research criteria at the time (1995 – 1999; Breitner et al., 1999).

In addition to the above procedures, a mail-in questionnaire was sent to participants who scored above 60 on the 3MS in order to obtain a measure of their level of physical activity, use of dietary supplements, and dietary intake (see "Diet" below). Follow-up examinations and interviews of surviving participants without dementia were conducted in 1998-1999 (Wave II), 2002 – 2004 (Wave III) and 2005 – 2007 (Wave IV). The current project utilized data from Wave 1 only (1995 – 1998). Inclusion criteria required that participants did not have dementia (based on their onset age) or

indeterminate cognitive status (did not complete appropriate dementia screening stages) at Wave 1.

Measures

Outcome Variable - Sleep Disturbance

A question on sleep disturbance, the outcome variable, was included as part of the health and medication sections of the interview. The self-report "yes/no" question, "Have you ever had sleep problems, insomnia?" was asked of each participant.

Predictor Variables

Genetic Variables

DNA from the buccal cells was processed using a polymerase chain reaction (Saunders et al., 1993) to determine the APOE genotype (Breitner et al., 1999). Genotyping for BDNF and related genes, including SNPs rs6265 (BDNF Val66Met), rs2289656 (BDNF receptor trkB), rs2072446 (BDNF receptor p75NTR), and rs56164415 (BDNF C270T), were assayed from blood DNA, or if unavailable, buccal DNA, using standard TaqMan Assays (Life Technologies; Matyi et al., 2017). Frequencies of major and minor alleles were examined to determine the representation of each SNP as homozygous for the major allele, heterozygous, and homozygous for the minor allele (i.e., AA, Aa, aa) as well as the presence or absence of the minor allele (i.e., AA vs. Aa or aa). Due to low counts of minor allele frequencies, all BDNF-related SNPs were categorized as a binary variable based on the presence or absence of the minor allele (e.g., Homozygous Major allele or Any Minor allele).

Physical Activity

Information on physical activity was collected using a mail-in, self-report questionnaire (see Appendix 1), that included three items regarding the frequency of light, moderate, and vigorous physical activity. The item querying light activity was asked as "About how many hours per day do you spend in light activity, such as walking, shopping, childcare, cooking, carrying light objects, cleaning, and repairing?"; moderate activity was asked as, "About how often do you take part in moderate physical activities including bowling, golf, light swimming, gardening, walks over 15 minutes, fishing, light bicycling, or other light sports?"; and vigorous activity was asked as, "About how often do you take part in vigorous physical activity including jogging, tennis, racquetball or squash, lap swimming, aerobics, vigorous bicycling, skiing, hiking, hunting or other vigorous sports?"

The type and amount of physical activity followed the procedures described in Sanders et al., (2020). Individuals who engaged in physical activity but who did not meet the moderate criteria were categorized as "light", and those who reported engaging in no physical activity were categorized as "sedentary". Thus, participants' engagement in physical activity was categorized as "sedentary," "light", "moderate", and "vigorous" levels. These levels were entered in statistical models as an ordinal variable.

Diet

Dietary intake was assessed by self-report mail-in questionnaire (see Appendix 1) using the Food Frequency Questionnaire (FFQ; Willett et al., 1985). The FFQ was developed from the Nurses' Health Study (Harvard FFQ; Willett et al., 1985) and has been validated as an appropriate measure for older adults (Munger et al., 1992). This measure requires participants to report the frequency of consumption for each of 142

food items. The frequency of consumption of macronutrients and total calories is available in the CCSMA dataset (Wengreen et al., 2013) based on the application of the Food Processor Program (ESHA Research, Portland, Oregon). The Food Processor Program is a large database containing nutrient information from the USDA Nutrient Composition Table and manufacturer information. A Mediterranean-style Diet adherence "score" was conceptualized following the procedures by Wengreen et al. (2013), which incorporated consumption of 8 major food groups including: high intakes of fruit, vegetables, whole grains, fish, beans, and ratio of monounsaturated fatty acids (MUFAs) to saturated fatty acids (SFAs) and low intakes of meat and meat products and high-fat dairy products. We conducted an energy adjustment for the frequency of intake for each food group following the procedure of Willett et al. (1985) to differentiate the specific nutrient intake adjusted for total caloric intake. The Mediterranean Diet scores were calculated by ranking the above individual food components for each participant, by the amount consumed relative to others in the sample (Wengreen, H., et al., 2013). For example, if the sample consisted of 1,000 subjects, those who had consumed the highest amount of fruit would be ranked as 1,000 for the fruit score, whereas those that consumed the lowest number of fruits would be ranked as 1. This process was repeated for each of the 8 components. Similar to Wengreen and colleagues (2013), the Mediterranean diet adherence scores were categorized into quintiles of the distribution which was used as an ordinal variable in statistical analyses.

In addition to the Mediterranean diet score, I also examined specific macronutrient intake that had previously been associated with sleep duration: proteins (Grandner et al., 2013), carbohydrates (Lindseth et al., 2013), and fats (Cao et al., 2016).

The amount of consumption of each macronutrient (e.g., protein, carbohydrate, fats), and alcohol was provided by the Food Processor program. Each macronutrient amount was then energy-adjusted using a method known as 'Nutrient Density' (Willett & Stampfer, 1986), in which each macronutrient intake (expressed in grams) was multiplied by the typical calories per gram for the macronutrient. The multiplier for macronutrient was as follows: proteins = 4, carbohydrates = 4, fats = 7, and alcohol = 9 (Cederbaum, 2012). Alcohol was converted to calories in order to obtain a total number of calories. The total calorie variable was created by summing the calories from each macronutrient plus alcohol. The final macronutrient variables were calculated as proportions of total calories by dividing each macronutrient intake by the total calorie intake. Total caloric intake was included as a covariate in all models exploring macronutrients.

Lastly, previous literature has suggested that flavonoids, a phytonutrient (plant chemical), are characterized as having strong antioxidant and anti-inflammatory properties (Kicinska & Jarmuszkiewicz, 2020). Flavonoid-rich foods are mostly found in fruits, vegetables, tea, and cocoa products (Godos et al., 2020) and flavonoids have been associated with improved sleep quality (Godos et al., 2020; Fernandez et al., 2003; Jiang et al., 2007). Flavonoids were measured in milligrams for the present study.

Covariates

APOE genotype (Blackman et al., 2022), age (Crowley, 2011), education (Krueger & Friedman, 2009), depression status (Yates et al., 2004), and body mass index (BMI; Romero-Corral et al., 2010) were used as covariates in analyses due to their associations with sleep disturbance. APOE genotype (described under "Genetic variables" was represented as a binary variable based on presence or absence of the minor

E4 allele. Age, sex and years of education were collected at the Wave 1 visit. Education was expressed as two groups: high school or less compared to more than high school, and depression status was categorized into individuals that were currently depressed versus not depressed.

Determination of depression status followed the procedures described by Steffens et al. (2000). Briefly, in the Wave 1 interview, participants were asked if they had ever experienced any of the following symptoms in their lifetime: depressed, sad, or blue mood; a loss of interest or pleasure; or irritability for nearly every day for 2 weeks or more. Those who did not endorse any of the symptoms and who were not taking any medications for depression were deemed "non-depressed." Participants who reported experiencing at least 1 of the 3 depression screening symptoms were asked follow-up questions adapted from the Diagnostic Interview Schedule (DIS; Robins et al., 1981). Participants also reported their age of onset of the earliest, the most recent, and the most severe depressive episodes. Criteria from the DSM-IV were used to classify individuals for all types of depression based on endorsement of the relevant symptoms including: depressed mood, reduced pleasure or interest, significant weight change (>5% body weight change in a month), insomnia or hypersomnia, psychomotor agitation, fatigue, or loss of energy, feelings of worthlessness or excessive guilt, difficulty with concentrating or thinking, or suicidal ideation (American Psychiatric Association, 2000). A classification of Consistent with the procedures by Steffens et al. (2000), major depression required at least 5 of these symptoms persisting for at least 2 weeks, and major depression with bereavement was assigned to those experiencing the depressive episode within the context of a death of a loved one (within 8 weeks of the depressive

episode). If participants did not meet major depression criteria but endorsed chronic (over 2 years) of significantly depressed mood and at least 2 of the depressive symptoms, they were classified as experiencing dysthymia. Individuals that endorsed some depressive symptoms but did not meet criteria for clinical depression were categorized as subclinical depressive disorders. From this group, subsyndromal depression was assigned to those who endorsed a minimum of 2 depressive symptoms (Steffens et al., 2000). Presence of bereavement was also accounted for (Steffens et al., 2000). Lastly, monosymptomatic depressed mood was designated if individuals experienced mood changes for longer than 2 weeks. For this study, participants who met criteria *for any type of current depression* (except for monosymptomatic depressed mood) were categorized as positive for currently depressed, and participants who did not meet the criteria for depression were categorized as not depressed. Depression status was used as a covariate in statistical analyses due to its association with sleep disturbance (Yates et al., 2004).

Body Mass Index (BMI) was calculated from self-report weight and height collected during the interview. BMI was calculated according to the formula by Keys et al. (1972):

$$\text{BMI} = \text{weight in kg} / \text{height in m}^2.$$

Four BMI groups were established according to BMI categories developed by the World Health Organization ([WHO]; 2000), and the National Heart, Lung, and Blood Institute ([NHLBI]; 1998): a value under 18.5 kg/m^2 was considered “under-weight,” 18.5–24.9 kg/m^2 was considered “normal weight,” 25.0–29.9 was considered “overweight,” and a BMI value over 30 was considered “obese”. These four groups were entered as an ordinal variable in statistical models. Note that new BMI guidelines have been created for

older adults (Winter et. al., 2014; Porter Starr & Bales, 2015), this study referred to the recommended BMI for the general population (adults 20 years old and older ([WHO]; 2000) as used in other studies of BMI and sleep disturbance (Gao et al., 2009; Romero-Corral et al., 2010; Schuld et al., 2000).

Data Analysis

To examine differences between those included vs. excluded in the analyses, t-test and chi-square tests were run on characteristics represented by continuous and categorical variables, respectively. Descriptive statistics were run on the final sample to examine differences by sex. Due to the differences between males and females on sleep disturbance (Krishnan & Collop, 2006), prior work showing sex differences on BDNF-related SNPs and significant dietary associations between husbands and wives within married couples in this sample, analyses were run separately for each sex. For each research question, logistic regression was conducted to investigate the association between each predictor variable of interest and sleep disturbance, the outcome variable. Model building proceeded sequentially, starting with a base model that examined the association of the primary predictor of interest (e.g., BDNF SNP) and the outcome (sleep disturbance). Next, covariates were entered sequentially, and the significance of interactions of interest was tested at each step. Variables or interactions were retained if the predictor reached a significance level of $p < .05$ or if their inclusion was theoretically important. Covariates tested included age, BMI, level of education, APOE ε4 allele, and depression. All analyses were performed using R software program (Version 1.4.1103). The analyses proceeded as follows for each research question:

For Research Question 1, the association between SNPs related to BDNF or its receptors and sleep disturbance was examined using separate logistic regression models for each primary predictor variable: rs6265 (BDNF Val66Met), rs2289656 (BDNF receptor trkB), rs2072446 (BDNF receptor p75NTR), and rs56164415 (BDNF C270T). The outcome variable was a report of sleep disturbance (presence/absence), and the covariates mentioned earlier were tested.

For Research Question 2, the association between lifestyle factors and sleep disturbance was examined using separate logistic regression models for each primary predictor variable: physical activity levels (sedentary, light, moderate, and vigorous), macronutrients (protein, fats, and carbohydrates), and Mediterranean diet adherence scores. The outcome variable was a report of sleep disturbance (yes/no), and the covariates mentioned earlier were tested.

For Research Question 3, the association between lifestyle factors and sleep disturbance was examined, considering moderation by SNPs related to BDNF or its receptors. Separate logistic regression models were run for each lifestyle factor from Research Question 2, and included interaction terms for each SNP (e.g., physical activity level x SNP) or dietary variable (e.g., Mediterranean quintile score x SNP). The outcome variable was a report of sleep disturbance (yes/no), and the covariates mentioned earlier were tested. The specific predictor variables, covariates, interactions, and outcome variables for each research question are presented in Appendix 1.

Results

Characteristics are provided for subsamples of the participants according to each research question. For research question 1 (examination of SNPs for BDNF or its

receptors and sleep disturbance), 4,427 met eligibility criteria. Specifically, of the initial 5,092 participants enrolled in the study, 359 were excluded for a positive dementia classification, 188 were excluded due to uncertain cognitive status from incomplete screening/assessment for dementia, and an additional 118 were excluded as their 3MS was at the cut-point for proxy report (60 or below). Of the eligible participants, 4,057 had data sufficient to be included in analyses. Incomplete cases were excluded sequentially due to missing data regarding the BDNF SNPs C270T (n = 272), sleep disturbance (n = 3), BMI (n = 76), APOE ε4 genotype (n = 17), and education (n = 2) (see Figure 1). Table 1 displays basic demographic and other characteristics that differed between those included vs. excluded from the analyses for research question 1.

For lifestyle-related investigations (research questions 2 and 3), the eligible sample was reduced to 3,575 from 4,427 due to 792 individuals lacking data on the FFQ and physical activity, and an additional 60 participants were excluded due to reports of extreme energy intakes ($\leq$ 500 or $\geq$ 5,000 calories per day). Out of the 3,575 eligible participants for lifestyle-related investigations, 216 individuals were further excluded due to missing data as follows: one missing data on sleep, an additional 118 were missing sufficient information on food intake to derive Mediterranean diet scores, an additional 5 were missing physical activity data, an additional 60 were missing BMI data, and an additional 32 were missing APOE ε4 genotype. The final sample size for research question 2, examining lifestyle factors and sleep disturbance was 3,359 (see Table 1). For research question 3, examining interactions between the SNPs and lifestyle factors, an additional 176 participants were excluded from the 3,359-sample size due to missing information on the BDNF SNP C270T (see Figure 1).

Table 1.

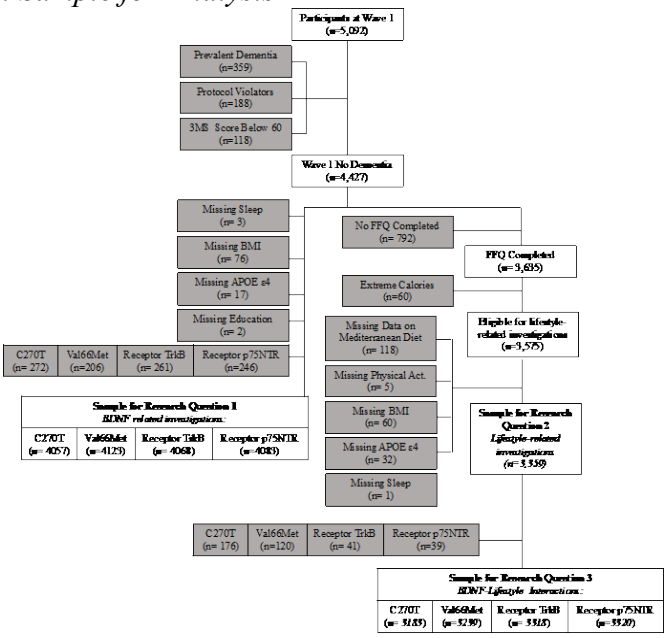
Participant Characteristics Comparing Those Included and Excluded in Analyses

	Inclusion Status for Analyses				
	Total Eligible (n = 4,427)	Excluded (n = 370)	Included (n = 4,057)	Test	P-Value
Sleep Disturbance				χ^2: 0.53	.467
No	2688 (60.7%)	230 (62.2%)	2458 (60.6%)		
Yes	1736 (39.2%)	137 (37%)	1599 (39.4%)		
Missing	3 (0.1%)	3 (0.8%)	0 (0%)		
C270T*				χ^2: 2.86	.091
Homozygous Major allele	3679 (83.1%)	81 (21.9%)	3598 (88.7%)		
Any Minor allele	476 (10.8%)	17 (4.6%)	459 (11.3%)		
Missing	272 (6.1%)	272 (73.5%)	0 (0%)		
Sex				χ^2: 2.46	.117
Male	1900 (42.9%)	144 (38.9%)	1756 (43.3%)		
Female	2527 (57.1%)	226 (61.1%)	2301 (56.7%)		
Missing	0 (0%)	0 (0%)	0 (0%)		
Depression				χ^2: 0.74	.391
No	4211 (95.1%)	501 (96%)	3710 (95%)		
Yes	216 (4.9%)	21 (4%)	195 (5%)		
Missing	0 (0%)	0 (0%)	0 (0%)		
Age in Years	74.86 (6.76)	75.68 (6.86)	74.78 (6.75)	t: 2.45	.014
Education, years				χ^2: 2.83	.092
HS or Less	2267 (51.2%)	204 (55.1%)	2063 (50.9%)		
More than HS	2157 (48.7%)	163 (44.1%)	1994 (49.1%)		
Missing	3 (0.1%)	3 (0.8%)	0 (0%)		
APOE e4 Alleles				χ^2: 3.24	.072
None	3042 (68.7%)	207 (55.9%)	2835 (69.9%)		
At Least One	1334 (30.1%)	112 (30.3%)	1222 (30.1%)		
Missing	51 (1.2%)	51 (13.8%)	0 (0%)		
Body Mass Index				χ^2: 3.03	.387
Underweight	103 (2.3%)	8 (2.2%)	95 (2.3%)		
Normal Weight	1754 (39.6%)	122 (33%)	1632 (40.2%)		
Overweight	1701 (38.4%)	119 (32.2%)	1582 (39%)		
Obese	790 (17.8%)	42 (11.4%)	748 (18.4%)		
Missing	79 (1.8%)	79 (21.4%)	0 (0%)		
Lifestyle Variables					
	Total (n = 3,575)	Excluded (n = 216)	Included (n = 3,359)	Test	P-Value
Physical Activity				χ^2: 5.44	.142
Sedentary	69 (1.9%)	8 (3.7%)	61 (1.8%)		
Light	976 (27.3%)	59 (27.3%)	917 (27.3%)		
Moderate	1996 (55.8%)	108 (50%)	1888 (56.2%)		
Vigorous	528 (14.8%)	35 (16.2%)	493 (14.7%)		
Missing	6 (0.2%)	6 (2.8%)	0 (0%)		
Mediterranean Diet Adherence Scores				χ^2: 2.14	.711
1st Quintile (1,903-11,766)	670 (18.7%)	14 (6.5%)	656 (19.5%)		
2nd Quintile (11,767-13,976)	688 (19.2%)	19 (8.8%)	669 (19.9%)		
3rd Quintile (13,977-15,926)	701 (19.6%)	22 (10.2%)	679 (20.2%)		
4th Quintile (15,927-18,264)	698 (19.5%)	23 (10.6%)	675 (20.1%)		
5th Quintile (18,265-29,427)	700 (19.6%)	20 (9.3%)	680 (20.2%)		
Missing	118 (3.3%)	10 (3.6%)	108 (3.3%)*		
Protein [M (SD) % of total calories]	16.86 (3.28)	16.90 (3.96)	16.86 (3.23)	t: 0.15	.879
Fats [M (SD) % of total calories]	29.58 (5.84)	29.28 (6.34)	29.60 (5.80)	t: -0.78	.436
Carbohydrates [M (SD) % of total calories]	53.19 (7.31)	53.62 (7.70)	53.16 (7.28)	t: 0.89	.373
Flavonoids [M (SD) mg]	14.22 (11.14)	12.75 (12.36)	14.31 (11.05)	t: -2.00	.045

Notes: For the table above, values are reported for BDNF C270T, which had the greatest number of missing genotypes (n=272). Other SNPs with missing data include: Val66Met (n=165; χ^2: 2.15, p = .142), Receptor p75NTR (n=177; χ^2: 0.39, p = .534), Receptor trkB (n=188; χ^2: 0.85, p = .356). APOE = apolipoprotein; HS = high school. *108 participants were lacking Mediterranean diet adherence scores due to missing data on food groups used to create the score, but they had sufficient data on other lifestyle variables and were thus included in the table.

Figure 1.

Flow Chart of Final Sample for Analysis



Notes: Flow chart of final sample for analysis. Participants included in analyses for models involving SNPs (n = 4,057), and Mediterranean diet (n = 3,359). Gray boxes represent subjects excluded from analyses at each level. 3MS = Modified Mini-Mental State Exam; FFQ = Food Frequency Questionnaire; Extreme Calories $\geq$ 500 or $\leq$5,000 calories per day.

Approximately 39% of the sample reported experiencing sleep disturbance. A significantly higher proportion of females (47% vs. 29.4% of males) and those with high school education or less reported experiencing sleep disturbance (41.7% vs. 37% of those greater than a high school education). Among those who endorsed current depression, 69.3% reported sleep disturbance, whereas 37.9% of those not depressed also reported sleep disturbance. A significantly higher proportion of individuals who reported sedentary and light physical activity also reported greater sleep disturbance (45.9% and 43.1%, respectively), compared to those who engaged in moderate and vigorous physical activity and endorsed sleep disturbance (38.2% and 33.4%, respectively). There were no significant associations between sleep disturbance and adherence to the Mediterranean diet or between sleep disturbance and SNPs for BDNF or BDNF receptors. See Table 2 for factors associated with sleep disturbance.

In examining sex differences and other participant characteristics, males were just slightly younger than females, and a greater proportion of females (63.7%) had completed less than a high school education than males (36.3%). Males and females engaged mostly moderate levels of physical activity (57.2% and 55.4%, respectively), however, 46.5% of males were considered to be overweight, while 43.6% of females were considered to be normal weight. Males had greater engagement in vigorous physical activity (57.8%) compared to females (42.2%). Significant differences were observed in adherence to the Mediterranean diet between males and females with lower adherence scores by males. Males had slightly higher mean scores for fats, while females had a slightly higher mean score for proteins, carbohydrates, and flavonoids. Table 3 displays sex differences among participant characteristics and lifestyle factors.

Table 2.

Overall Baseline Characteristics by Sleep Disturbance

		Sleep Disturbance			
	Total (n = 4,057)	No (n = 2,458)	Yes (n = 1,599)	Test Statistic	P-Value
Val66Met				χ^2: 0.14	.705
Homozygous Major allele	2640 (100%)	1592 (60.3%)	1048 (39.7%)		
Any Minor allele	1376 (100%)	839 (61%)	537 (39%)		
Receptor p75NTR				χ^2: 2.25	.134
Homozygous Major allele	3656 (100%)	2204 (60.3%)	1452 (39.7%)		
Any Minor allele	331 (100%)	214 (64.7%)	117 (35.3%)		
Receptor trkB				χ^2: 0.11	.739
Homozygous Major allele	2658 (100%)	1600 (60.2%)	1058 (39.8%)		
Any Minor allele	1324 (100%)	805 (60.8%)	519 (39.2%)		
C270T,				χ^2: 1.15	.283
Homozygous Major allele	3598 (100%)	2191 (60.9%)	1407 (39.1%)		
Any Minor allele	459 (100%)	267 (58.2%)	192 (41.8%)		
Sex				χ^2: 128.18	<.001
Male	1756 (100%)	1239 (70.6%)	517 (29.4%)		
Female	2301 (100%)	1219 (53%)	1082 (47%)		
Age	74.78 (6.75)	74.69 (6.70)	74.93 (6.82)	t: -1.12	.261
Depression				χ^2: 77.21	<.001
No	3858 (100%)	2397 (62.1%)	1461 (37.9%)		
Yes	199 (100%)	61 (30.7%)	138 (69.3%)		
Education, years				χ^2: 9.28	.002
HS or less	2063 (100%)	1202 (58.3%)	861 (41.7%)		
More than HS	1994 (100%)	1256 (63%)	738 (37%)		
APOE ε4 Alleles				χ^2: 1.44	.23
None	2835 (100%)	1700 (60%)	1135 (40%)		
At Least One	1222 (100%)	758 (62%)	464 (38%)		
Body Mass Index				χ^2: 3.75	.289
Underweight	95 (100%)	59 (62.1%)	36 (37.9%)		
Normal Weight	1632 (100%)	985 (60.4%)	647 (39.6%)		
Overweight	1582 (100%)	981 (62%)	601 (38%)		
Obese	748 (100%)	433 (57.9%)	315 (42.1%)		
Lifestyle Variables		Sleep Disturbance			
	Total (n = 3,359)	No (n = 2,052)	Yes (n = 1,307)	Test Statistic	P-Value
Physical Activity				χ^2: 15.45	.001
Sedentary	61 (100%)	33 (54.1%)	28 (45.9%)		
Light	917 (100%)	522 (56.9%)	395 (43.1%)		
Moderate	1888 (100%)	1167 (61.8%)	721 (38.2%)		
Vigorous	493 (100%)	330 (66.9%)	163 (33.1%)		
Mediterranean Diet Adherence Scores				χ^2: 4.78	.311
1st Quintile (1,903-11,766)	656 (100%)	405 (61.7%)	251 (38.3%)		
2nd Quintile (11,767-13,976)	669 (100%)	426 (63.7%)	243 (36.3%)		
3rd Quintile (13,977-15,926)	679 (100%)	406 (59.8%)	273 (40.2%)		
4th Quintile (15,927-18,264)	675 (100%)	418 (61.9%)	257 (38.1%)		
5th Quintile (18,265-29,427)	680 (100%)	397 (58.4%)	283 (41.6%)		
Protein [M (SD) % of total calories]	16.86 (3.23)	16.84 (3.21)	16.89 (3.26)	t: -0.42	.674
Fats [M (SD) % of total calories]	29.60 (5.80)	29.78 (5.66)	29.31 (6.01)	t: 2.25	.025
Carbohydrates [M (SD) % of total calories]	53.16 (7.28)	52.94 (7.19)	53.52 (7.41)	t: -2.23	.026
Flavonoids [M (SD) mg]	14.31 (11.05)	14.37 (10.86)	14.22 (11.36)	t: 0.39	.693

Table 3.

Baseline Characteristics Stratified by Sex

		Sex			
	Total (n = 4057)	Male (n = 1756)	Female (n = 2301)	Test	P-Value
Val66Met				χ^2: 0.92	.337
Homozygous Major allele	2640 (100%)	1156 (43.8%)	1484 (56.2%)		
Any Minor allele	1376 (100%)	580 (42.2%)	796 (57.8%)		
Receptor p75NTR				χ^2: 0.22	.64
Homozygous Major allele	3656 (100%)	1580 (43.2%)	2076 (56.8%)		
Any Minor allele	331 (100%)	148 (44.7%)	183 (55.3%)		
Receptor trkB				χ^2: 2.59	.107
Homozygous Major allele	2658 (100%)	1175 (44.2%)	1483 (55.8%)		
Any Minor allele	1324 (100%)	549 (41.5%)	775 (58.5%)		
C270T				χ^2: 0.01	.907
Homozygous Major allele	3598 (100%)	1559 (43.3%)	2039 (56.7%)		
Any Minor allele	459 (100%)	197 (42.9%)	262 (57.1%)		
Age	74.78 (6.75)	74.21 (6.61)	75.22 (6.82)	t: -4.74	<.001
Depression				χ^2: 12.02	<.001
No	3858 (100%)	1694 (43.9%)	2164 (56.1%)		
Yes	199 (100%)	62 (31.2%)	137 (68.8%)		
Education, years				χ^2: 82.65	<.001
HS or less	2063 (100%)	749 (36.3%)	1314 (63.7%)		
More than HS	1994 (100%)	1007 (50.5%)	987 (49.5%)		
APOE ε4 Alleles				χ^2: 0.15	.700
None	2835 (100%)	1221 (43.1%)	1614 (56.9%)		
At Least One	1222 (100%)	535 (43.8%)	687 (56.2%)		
Body Mass Index				χ^2: 90.87	<.001
Underweight	95 (2.3%)	16 (0.9%)	79 (3.4%)		
Normal Weight	1632 (40.2%)	629 (35.8%)	1003 (43.6%)		
Overweight	1582 (39%)	816 (46.5%)	766 (33.3%)		
Obese	748 (18.4%)	295 (16.8%)	453 (19.7%)		
Lifestyle Variables					
	Total (n = 3,359)	Male (n = 1,459)	Female (n =1,900)	Test	P-Value
Physical Activity				χ^2: 73.63	<.001
Sedentary	61 (1.8%)	25 (1.7%)	36 (1.9%)		
Light	917 (27.3%)	314 (21.5%)	603 (31.7%)		
Moderate	1888 (56.2%)	835 (57.2%)	1053 (55.4%)		
Vigorous	493 (14.7%)	285 (19.5%)	208 (10.9%)		
Mediterranean Diet Adherence Scores				χ^2: 61.12	<.001
1st Quintile (1,903-11,766)	656 (100%)	349 (53.2%)	307 (46.8%)		
2nd Quintile (11,767-13,976)	669 (100%)	322 (48.1%)	347 (51.9%)		
3rd Quintile (13,977-15,926)	679 (100%)	298 (43.9%)	381 (56.1%)		
4th Quintile (15,927-18,264)	675 (100%)	250 (37%)	425 (63%)		
5th Quintile (18,265-29,427)	680 (100%)	240 (35.3%)	440 (64.7%)		
Protein [M (SD) % of total calories]	16.86 (3.23)	16.56 (3.03)	17.09 (3.36)	t: -4.72	<.001
Fats [M (SD) % of total calories]	29.60 (5.80)	30.32 (5.69)	29.04 (5.83)	t: 6.34	<.001
Carbohydrates [M (SD) % of total calories]	53.16 (7.28)	52.48 (7.28)	53.69 (7.24)	t: -4.82	<.001
Flavonoids [M (SD) mg]	14.31 (11.05)	13.34 (9.89)	15.06 (11.82)	t: -4.6	<.001

Logistic Regression Models

SNPs for BDNF or its receptors and Sleep Disturbance

Separate binary logistic regression models examined the association of individual BDNF/ receptor-related SNPs and sleep disturbance for each sex. In males, presence of the minor allele of each SNP was not significantly associated with sleep disturbance compared to the homozygous major alleles: Val66Met (OR = 1.08, 95% CI = [0.87; 1.34], p = .479), Receptor p75NTR (OR = 0.73, 95% CI = [0.49; 1.06], p = .105), Receptor trkB (OR = 0.88, 95% CI = [0.70; 1.10], p = .266) , C270T (OR = 1.17, 95% CI = [0.85; 1.60], p = .34). Similar results were obtained among females: Val66Met (OR = 0.90, 95% CI = [0.76; 1.07], p = .216), Receptor p75NTR (OR = 0.94, 95% CI = [0.69; 1.27], p = .665), Receptor trkB (OR = 1.01, 95% CI = [0.85; 1.20], p = .919), C270T (OR = 1.07, 95% CI = [0.83; 1.39], p = .59). The results did not change with the inclusion of significant covariates (see tables 4 and 5 for the covariate-adjusted models). Depression had a three-fold increase in the odds of sleep disturbance; no other covariate was significant in any of the models examining BDNF-related SNPs.

Table 4.

Males: Fully adjusted Logistic Regression Models Comparisons for Sleep Disturbance Associated with BDNF-related SNPs

	Val66Met		Receptor $p75^{NTR}$		Receptor trkB		C270T	
Variable	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.
(Intercept)	0.40 [0.35; 0.45]	<.001***	0.46 [0.37; 0.58]	<.001***	0.43 [0.38; 0.48]	<.001***	0.40 [0.36; 0.45]	<.001***
Depression	3.74 [2.25; 6.33]	<.001***	3.75 [2.25; 6.35]	<.001***	3.74 [2.24; 6.33]	<.001***	3.63 [2.17; 6.16]	<.001***
Val66Met[a]	1.08 [0.87; 1.34]	.479	------		------		------	
Receptor $p75^{NTRa}$	------		0.73 [0.49; 1.06]	.105	------		------	
Receptor trkB[a]	------		------		0.88 [0.70; 1.10]	.266	------	
C270T[a]	------		------		------		1.17 [0.85; 1.60]	.34
Sample size	1781		1764		1761		1756	

*** p < 0.001; ** p < 0.01; *p < 0.05; [a] Any Minor allele

Table 5.

Females: Fully adjusted Logistic Regression Models Comparisons for Sleep Disturbance Associated with BDNF SNPs

	Val66Met		Receptor $p75^{NTR}$		Receptor trkB		C270T	
Variable	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.
(Intercept)	0.87 [0.78; 0.96]	.004**	0.83 [0.76; 0.91]	<.001***	0.83 [0.75; 0.92]	<.001***	0.83 [0.76; 0.90]	<.001***
Currently Depressed	3.52 [2.40; 5.29]	<.001***	3.45 [2.37; 5.16]	<.001***	3.48 [2.38; 5.23]	<.001***	3.36 [2.30; 5.03]	<.001***
Val66Met[a]	0.90 [0.76; 1.07]	.216	------		------		------	
Receptor $p75^{NTRa}$	------		0.94 [0.69; 1.27]	0.665	------		------	
Receptor trkB[a]	------		------		1.01 [0.85; 1.20]	0.919	------	
C270T[a]	------		------		------		1.07 [0.83; 1.39]	0.59
Sample size	2342		2319		2307		2301	

*** p < 0.001; ** p < 0.01; *p < 0.05; [a] Any Minor allele

Lifestyle Factors

Physical Activity

Among males, increasing level of physical activity (sedentary, light, moderate and vigorous) was significantly associated with a lower risk of sleep disturbance (OR = 0.82, 95% CI = [0.70; 0.96]; p = .015), and remained significant with the inclusion of covariates (OR = 0.82, 95% CI = [0.71; 0.97]; p =.023). Thus, engagement in each higher level of physical activity was associated with an 18% reduction in odds of sleep disturbance in males. Among females however, level of physical activity was not associated with sleep disturbance in either bivariate (p = .145) or covariate-adjusted models (p = .343). Again, only depression was associated with sleep disturbance in both males and females. See Tables 6 and 7 for the results of physical activity and sleep disturbance in males and females, respectively.

Mediterranean Diet

Adherence to the Mediterranean diet was associated with increased odds of sleep disturbance in males, (OR = 1.08, 95% CI = [1.00; 1.18]; p = .047), but not in females (p = .227). With each increase in quintile (of adherence) to the Mediterranean diet, male participants had an 8% higher odds of reporting sleep disturbance. Again, depression was associated with more than a three-fold increase in risk of sleep disturbance; no other covariates were significant.

Macronutrients

For males, consumption of proteins (OR = 1.01, 95% CI = [0.98; 1.05]; p = .428), carbohydrates (OR = 1.01, 95% CI = [0.99; 1.02]; p = .261) or fats (OR = 0.98, 95% CI = [0.97; 1.00]; p = .116) was not significantly associated with sleep disturbance in bivariate

or covariate-adjusted models. Results were similar among females: proteins (OR = 0.99, 95% CI = [0.97; 1.02]; p = .622), carbohydrates (OR = 1.01, 95% CI = [0.99; 1.02]; p = .348) and fats (OR = 1.00, 95% CI = [0.98; 1.01]; p =.619) (see Table 6 and Table 7). Again, depression was associated with more than a three-fold increase in risk of sleep disturbance in both males and females; no other covariates were significantly associated with sleep disturbance.

Flavonoids

Consumption of flavonoids was not significantly associated with sleep disturbance in bivariate or covariate-adjusted models in males (OR = 1.01, 95% CI = [0.99; 1.02]; p = .396) or in females (OR = 0.99, 95% CI = [0.98; 1.00]; p = .059). Again, amongst covariates, depression was associated with three times the odds of sleep disturbance in both males and females. No other covariate was found to be significantly associated with sleep disturbance. Tables 6 and 7 display the results of the final analyses for research question 2.

Table 6.

Males: Covariate-adjusted Logistic Regression Models for Sleep Disturbance Associated with Lifestyle factors

	Physical Activity		Mediterranean Diet		Protein		Fats		Carbohydrates		Flavonoids	
Variable	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.
(Intercept)	0.66 [0.38; 1.14]	.138	0.31 [0.20; 0.47]	<.001***	0.30 [0.15; 0.61]	<.001***	0.61 [0.32; 1.17]	.136	0.24 [0.10; 0.58]	.002**	0.39 [0.28; 0.53]	<.001***
Depression	3.24 [1.78; 6.01]	<.001***	3.59 [1.98; 6.64]	<.001***	3.38 [1.86; 6.25]	<.001***	3.34 [1.84; 6.19]	<.001***	3.34 [1.84; 6.19]	<.001***	3.36 [1.85; 6.22]	<.001***
Total Calories[a]	1.00 [1.00; 1.00]	.653	1.00 [1.00; 1.00]	.745	------		------		------		------	
Nutrient Calories[b]	------		------		1.00 [1.00; 1.00]	.659	1.00 [1.00; 1.00]	.602	1.00 [1.00; 1.00]	.663	1.00 [1.00; 1.00]	.928
Physical Activity	0.83 [0.71; 0.97]	.023*	------		------		------		------		------	
Mediterranean Diet	------		1.08 [1.00; 1.18]	.047*	------		------		------		------	
Proteins	------		------		1.01 [0.98; 1.05]	.428	------		------		------	
Fats	------		------		------		0.98 [0.97; 1.00]	.116	------		------	
Carbohydrates	------		------		------		------		1.01 [0.99; 1.02]	.261	------	
Flavonoids	------		------		------		------		------		1.01 [0.99; 1.02]	.396
Sample size	1500		1489		1501		1501		1501		1501	

[a] Total Calories used for the Mediterranean models. [b] Nutrient Calories accounted for alcohol in macronutrients and flavonoids.

Table 7.

Females: Covariate-adjusted Logistic Regression Models for Sleep Disturbance Associated with Lifestyle factors

	Physical Activity		Mediterranean Diet		Protein		Fats		Carbohydrates		Flavonoids	
Variable	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.
(Intercept)	0.98 [0.64; 1.51]	.923	0.97 [0.69; 1.36]	.871	0.92 [0.55; 1.53]	.737	0.91 [0.56; 1.49]	.708	0.59 [0.29; 1.21]	.153	0.82 [0.64; 1.05]	.12
Depression	3.02 [1.95; 4.81]	<.001***	3.15 [2.02; 5.04]	<.001***	3.08 [1.99; 4.90]	<.001***	3.08 [1.99; 4.90]	<.001***	3.09 [2.00; 4.92]	<.001***	3.08 [1.99; 4.90]	<.001***
Total Calories[a]	1.00 [1.00; 1.00]	.946	1.00 [1.00; 1.00]	.569	------		------		------		------	
Nutrient Calories[b]	------		------		1.00 [1.00; 1.00]	0.855	1.00 [1.00; 1.00]	0.908	1.00 [1.00; 1.00]	0.913	1.00 [1.00; 1.00]	.395
Physical Activity	0.94 [0.82; 1.07]	.343	------		------		------		------		------	
Mediterranean Diet	------		0.96 [0.90; 1.02]	.227	------		------		------		------	
Proteins	------		------		0.99 [0.97; 1.02]	.622	------		------		------	
Fats	------		------		------		1.00 [0.98; 1.01]	.619	------		------	
Carbohydrates	------		------		------		------		1.01 [0.99; 1.02]	.348	------	
Flavonoids	------		------		------		------		------		0.99 [0.98; 1.00]	.059
Sample size	1973		1967		1978		1978		1978		1978	

[a] Total Calories used for the Mediterranean models. [b] Nutrient Calories accounted for alcohol in macronutrients and flavonoids.

Interactions: SNPs for BDNF or its receptors and Lifestyle Factors in their Association with Sleep Disturbance

SNPs and Physical Activity

An examination of the interactions between individual BDNF/receptor-related SNPs and physical activity yielded no significant associations with sleep disturbance in either sex, with the inclusion of depression as a covariate. Among males, the interaction p-values were as follows: Val66Met (interaction p = .899), Receptor p75NTR (interaction p = .375), Receptor trkB (interaction p = .314), C270T (interaction p = .44). See Table 8 for model parameter estimates for males.

Among females there was a trend for an interaction between Val66Met and physical activity (interaction p = .088). Prior to examining the nature of the interaction, levels of physical activity were recategorized due to low cell sizes (only 16 females with the minor allele who were sedentary) into a dichotomous set of levels (sedentary/light, and moderate/vigorous). Among females with a Val66Met minor allele, those with sedentary/light physical activity had a 45% increased risk of sleep disturbance compared to those with moderate/vigorous physical activity (OR = 1.45, 95% CI = [1.05; 2.00]; p = .026). None of the interactions between the other SNPS and physical activity were significant: Receptor p75NTR (interaction p = .610), Receptor trkB (interaction p = 0.678), C270T (interaction p = .235). See Table 9 for model parameter estimates for females.

SNPs and Mediterranean Diet and Other Dietary Variables

No significant associations were found with sleep disturbance in the interactions between individual BDNF/receptor-related SNPs and the Mediterranean Diet adherence

scores in males: Val66Met (interaction p = .954), Receptor p75NTR (interaction p = .719), Receptor trkB (interaction p =.340) , C270T (interaction p =.789), or in females: Val66Met (interaction p = .767), Receptor p75NTR (interaction p = .727), Receptor trkB (interaction p = .707) , C270T (interaction p =.674). No significant interactions were found between the BDNF receptor-related SNPs and the amount of consumption of protein, carbohydrates, fats or flavonoids and sleep disturbance for males or females. Tables 8 and 9 display the covariate-adjusted analyses for research question 3.

Table 8.

Males: Covariate-adjusted Logistic Regression Models for Interactions between BDNF receptor-related SNPs and Lifestyle Factors on Sleep Disturbance

	Val66Met		Receptor $p75^{NTR}$		Receptor trkB		C270T	
Variable	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.
(Intercept)	0.89 [0.75; 1.05]	<.001 ***	0.45 [0.36; 0.57]	<.001 ***	0.46 [0.36; 0.58]	<.001 ***	0.43 [0.34; 0.54]	<.001 ***
Depression	3.50 [2.38; 5.25]	<.001 ***	3.61 [2.16; 6.14]	<.001 ***	3.61 [2.16; 6.14]	<.001 ***	3.62 [2.16; 6.15]	<.001 ***
Total Calories[a]	1.00 [1.00; 1.00]	.897	1.00 [1.00; 1.00]	.827	1.00 [1.00; 1.00]	.689	3.27 [1.78; 6.10]	.659
SNP	1.06 [0.38; 2.98]	.907	0.39 [0.07; 2.16]	.290	1.53 [0.53; 4.46]	.43	2.03 [0.48; 8.71]	.337
Physical Activity	0.80 [0.65; 0.98]	.023 *	0.79 [0.66; 0.94]	.007 **	0.86 [0.70; 1.05]	.128	0.82 [0.69; 0.98]	.029 *
Physical Activity*SNP	0.98 [0.69; 1.38]	.899	1.29 [0.74; 2.27]	.375	0.83 [0.58; 1.19]	.314	0.83 [0.50; 1.34]	.44
SNP	1.04 [0.60; 1.79]	.899	0.68 [0.26; 1.72]	.433	0.68 [0.38; 1.21]	.193	1.21 [0.54; 2.60]	.635
Medi	1.08 [0.98; 1.20]	.130	1.09 [1.00; 1.18]	.060	1.06 [0.96; 1.17]	.231	1.09 [1.00; 1.19]	.049 *
Medi*SNP	0.99 [0.84; 1.18]	.954	1.05 [0.79; 1.41]	.719	1.09 [0.91; 1.31]	.34	0.97 [0.75; 1.24]	.789
Nutrient Calories[b]	1.00 [1.00; 1.00]	.841	1.00 [1.00; 1.00]	.749	1.00 [1.00; 1.00]	.631	1.00 [1.00; 1.00]	.648
SNP	0.74 [0.20; 2.75]	.651	0.34 [0.04; 2.84]	.322	0.77 [0.20; 2.98]	.705	1.44 [0.22; 9.28]	.703
Protein	1.00 [0.96; 1.05]	.899	1.01 [0.97; 1.05]	.74	1.01 [0.97; 1.06]	.624	1.02 [0.98; 1.06]	.432
Protein*SNP	1.02 [0.94; 1.10]	.631	1.05 [0.93; 1.20]	.407	1.01 [0.93; 1.09]	.836	0.99 [0.88; 1.10]	.822
Nutrient Calories	1.00 [1.00; 1.00]	.83	1.00 [1.00; 1.00]	.767	1.00 [1.00; 1.00]	.637	1.00 [1.00; 1.00]	.658
SNP	1.34 [0.24; 7.57]	.738	0.26 [0.02; 3.58]	.33	1.06 [0.17; 6.45]	.949	0.98 [0.07; 13.04]	.989
Carbs	1.01 [0.99; 1.03]	.296	1.01 [0.99; 1.02]	.447	1.01 [0.99; 1.03]	.375	1.01 [0.99; 1.02]	.374
Carbs*SNP	0.99 [0.96; 1.03]	.744	1.02 [0.97; 1.07]	.402	1.00 [0.96; 1.03]	.85	1.00 [0.96; 1.05]	.896
Nutrient Calories	1.00 [1.00; 1.00]	.775	1.00 [1.00; 1.00]	.678	1.00 [1.00; 1.00]	.582	1.00 [1.00; 1.00]	.602
SNP	0.97 [0.27; 3.46]	.959	2.42 [0.38; 15.44]	.347	0.72 [0.18; 2.86]	.646	1.37 [0.21; 8.88]	.74
Fats	0.99 [0.96; 1.01]	.257	0.99 [0.97; 1.01]	.312	0.99 [0.96; 1.01]	.202	0.99 [0.97; 1.01]	.207
Fats*SNP	1.00 [0.96; 1.04]	.944	0.96 [0.90; 1.02]	.234	1.01 [0.96; 1.05]	.767	0.99 [0.93; 1.06]	.858
Nutrient Calories	1.00 [1.00; 1.00]	.819	1.00 [1.00; 1.00]	.814	1.00 [1.00; 1.00]	.979	1.00 [1.00; 1.00]	.947
SNP	1.26 [0.85; 1.88]	.252	0.97 [0.48; 1.92]	.932	0.79 [0.52; 1.21]	.280	1.62 [0.92; 2.87]	.095
Flavonoids	1.01 [1.00; 1.03]	.157	1.01 [0.99; 1.02]	.252	1.00 [0.99; 1.02]	.703	1.01 [1.00; 1.02]	.155
Flavonoids*SNP	0.98 [0.96; 1.01]	.167	0.99 [0.95; 1.02]	.532	1.01 [0.98; 1.04]	.501	0.98 [0.94; 1.01]	.157
Sample size.	1408		1396		1392		1387	

[a] Total Calories used for the Mediterranean models. [b] Nutrient Calories accounted for alcohol in macronutrients and flavonoid models.

Table 9.

Females: Covariate-adjusted Logistic Regression Models for Interactions between BDNF receptor-related SNPs and Lifestyle Factors on Sleep Disturbance

	Val66Met		Receptor $p75^{NTR}$		C270T			
Variable	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.	OR [CI]	Sig.
(Intercept)	1.06 [0.72; 1.56]	.785	1.09 [0.76; 1.56]	.649	1.03 [0.70; 1.51]	.898	1.04 [0.73; 1.49]	.825
Depression	3.02 [1.91; 4.94]	<.001***	2.93 [1.85; 4.75]	<.001***	3.03 [1.91; 4.95]	<.001***	2.91 [1.84; 4.72]	<.001***
Total Calories[a]	1.00 [1.00; 1.00]	.415	1.00 [1.00; 1.00]	.788	1.00 [1.00; 1.00]	.358	1.00 [1.00; 1.00]	.492
SNP	1.06 [0.38; 2.98]	.155	1.79 [0.80; 3.99]	.728	0.85 [0.38; 1.93]	.704	2.00 [0.61; 6.73]	.256
Physical Activity	0.80 [0.65; 0.98]	.727	1.03 [0.87; 1.22]	.421	0.92 [0.77; 1.09]	.324	0.98 [0.84; 1.13]	.751
Physical Activity*SNP	0.78 [0.59; 1.04]	.088	1.29 [0.74; 2.27]	.610	1.06 [0.80; 1.42]	.678	0.77 [0.50; 1.18]	.235
SNP	0.97 [0.60; 1.57]	.89	0.79 [0.33; 1.86]	.596	1.11 [0.67; 1.82]	.687	0.83 [0.40; 1.72]	.616
Medi	0.97 [0.89; 1.05]	.417	0.94 [0.88; 1.01]	.111	0.97 [0.89; 1.05]	.396	0.95 [0.89; 1.03]	.203
Medi*SNP	0.98 [0.85; 1.13]	.767	1.04 [0.82; 1.33]	.727	0.97 [0.84; 1.12]	.707	1.05 [0.85; 1.29]	.674
Nutrient Calories[b]	1.00 [1.00; 1.00]	.984	1.00 [1.00; 1.00]	.827	1.00 [1.00; 1.00]	.712	1.00 [1.00; 1.00]	.695
SNP	0.51 [0.19; 1.34]	.173	0.43 [0.08; 2.34]	.333	0.58 [0.21; 1.58]	.284	0.37 [0.08; 1.73]	.208
Protein	0.98 [0.95; 1.01]	.272	0.99 [0.96; 1.02]	.497	0.98 [0.95; 1.02]	.3	0.99 [0.96; 1.01]	.318
Protein*SNP	1.03 [0.98; 1.09]	.229	1.04 [0.95; 1.15]	.398	1.03 [0.97; 1.09]	.276	1.06 [0.97; 1.16]	.203
Nutrient Calories	1.00 [1.00; 1.00]	.989	1.00 [1.00; 1.00]	.886	1.00 [1.00; 1.00]	.734	1.00 [1.00; 1.00]	.838
SNP	2.41 [0.60; 9.78]	.216	2.44 [0.22; 28.15]	.465	2.04 [0.49; 8.40]	.325	0.63 [0.07; 5.76]	.683
Carbs	1.01 [1.00; 1.03]	.115	1.01 [1.00; 1.02]	.205	1.01 [1.00; 1.03]	.132	1.01 [0.99; 1.02]	.345
Carbs*SNP	0.98 [0.96; 1.01]	.168	0.98 [0.94; 1.03]	.401	0.99 [0.96; 1.01]	.317	1.01 [0.97; 1.05]	.686
Nutrient Calories	1.00 [1.00; 1.00]	0.979	1.00 [1.00; 1.00]	0.885	1.00 [1.00; 1.00]	0.727	1.00 [1.00; 1.00]	.847
SNP	0.58 [0.23; 1.48]	.256	0.47 [0.09; 2.41]	.366	0.73 [0.28; 1.88]	.515	2.40 [0.54; 11.00]	.253
Fats	0.99 [0.97; 1.01]	.317	0.99 [0.98; 1.01]	.365	0.99 [0.97; 1.01]	.324	1.00 [0.98; 1.01]	.791
Fats*SNP	1.02 [0.98; 1.05]	.336	1.02 [0.97; 1.08]	.439	1.01 [0.98; 1.04]	.511	0.97 [0.92; 1.02]	.24
Nutrient Calories	1.00 [1.00; 1.00]	.405	1.00 [1.00; 1.00]	0.586	1.00 [1.00; 1.00]	0.578	1.00 [1.00; 1.00]	.579
SNP	0.93 [0.69; 1.27]	.663	0.84 [0.49; 1.42]	.512	0.85 [0.62; 1.17]	.325	0.93 [0.59; 1.47]	.766
Flavonoids	0.99 [0.98; 1.00]	.176	0.99 [0.98; 1.00]	.121	0.99 [0.98; 1.00]	.032*	0.99 [0.98; 1.00]	.085
Flavonoids*SNP	1.00 [0.98; 1.01]	.789	1.00 [0.98; 1.03]	.724	1.01 [0.99; 1.03]	.216	1.00 [0.98; 1.03]	.751
Sample size.	1784		1814		1818		1799	

[a] Total Calories used for the Mediterranean models. [b] Nutrient Calories accounted for alcohol in macronutrients and flavonoid models.

Discussion

The purpose of this study was to examine the associations between selected BDNF-related SNPs, and lifestyle factors of physical activity and diet and their interactions on the risk for sleep disturbance in older adults. BDNF-related SNPs were not significantly associated with sleep disturbance in either males or females. Among lifestyle factors, males exhibited reduced risk for sleep disturbance with increasing engagement in physical activity but increased sleep disturbance with greater adherence to the Mediterranean diet. Finally, SNPs for BDNF or its receptors did not significantly interact with lifestyle factors and sleep disturbance, except among females there was a trend for minor allele carriers of Val66Met reporting sedentary-to-light physical activity exhibiting increased risk of sleep disturbance compared to those reporting moderate-to-vigorous physical activity.

Very few studies have examined BDNF-related genes and their relationship with sleep disturbance. Some research has been conducted with one SNP, Val66Met and some aspects of sleep. For example, there was no association between Val66Met genotypes with report of subjective sleepiness (e.g., general experiences of sleepiness at a given moment) in younger adults (Bachmann et al., 2012). However, one study reported Val66Met homozygotes (Val/Val) exhibited increased EEG slow wave activity (an established physiological marker of sleep need in the early stages of NREM sleep) compared to Val66Met heterozygotes (Bachmann et al., 2012). It may be possible that BDNF SNPs are associated with specific sleep characteristics rather than global aspects of sleep.

However, previous studies have suggested that circulating BDNF (measured in blood serum) plays a role in insomnia (Giese et al., 2014) or sleep duration (Fan et al., 2019). Specifically, in middle-aged study participants, lower serum BDNF levels were associated with insomnia (Giese et al., 2014), and in another study, middle-aged adults with short sleep duration (sleep time < 6 hours) had lower serum BDNF levels compared to those with normal sleep duration (sleep time ≥ 6 hours; Fan et al., 2019). Serum BDNF levels were not available in the Cache County Study sample and thus the current results cannot be directly compared to these studies. Additionally, the above studies were conducted with middle-aged adults, whereas my study sample consisted of individuals above age 65. Age differences may be notable as prevalence rates of insomnia are higher (Kocevska et al., 2021) and BDNF levels are lower (Erickson et al., (2010) in older adults. Additionally, the relationship between BDNF genotypes and peripheral BDNF levels is unclear. For example, val66MET genotypes have not been associated with peripheral BDNF levels in previous studies [see study and meta-analysis by Terracciano and colleagues (Terracciano et al., 2013)].

Physical Activity

Previous studies in older adults have suggested a significant association between physical activity (regardless of intensity) and sleep efficiency and duration (Dolezal, B. A., et. al., 2017; Du, S., et. al., 2015). In a systematic review by the Du et al. (2015), researchers analyzed five randomized controlled trials that examined Tai Chi as the main intervention for improving sleep quality in older adults (Du et al., 2015). All of these studies reported that Tai Chi exercise had a significant effect in improving subjective sleep ratings in older adults on the Pittsburgh Sleep Quality Index (Irwin et al., 2008; Li

et al., 2004; Liu and Yao, 2010) or on a global measure of sleep quality (Hosseini et al., 2011; Nguyen and Kruse 2012).

In the current study, increasing levels of physical activity was associated with reduced sleep disturbance, but only among men. Sex differences observed here may reflect the overall lower frequency of moderate/vigorous physical activity among females in this sample or the potential moderating role of BDNF-related SNPs (see interaction discussed below). While sex differences and BDNF levels have not been well-researched, one study suggested that physical activity raises BDNF levels more prominently in young adult males than females (Dinoff et al., 2017). Future research on sex differences is needed to explore whether such differences exist for older adults and the intensity or type of physical activity that may promote BDNF secretion.

Diet

Previous studies on the Mediterranean diet and sleep have found that greater adherence was associated with greater sleep quality and quantity (Campanini et al., 2017; Mamalaki et al., 2018). Specifically, among non-institutionalized older adults living in Spain, greater adherence to the Mediterranean diet was associated with a 32% lower risk of poor sleep quality (Campanini et al., 2017). Similarly, in a population-based study of older adults residing in France, for each one-unit increase in the Mediterranean diet score, there was an estimated 8.7% increase in sleep quality (Mamalaki et al., 2018). In the current study, unexpectedly, greater adherence to the Mediterranean diet was associated with *increased* sleep disturbance in males only. It is unclear what underlies this association, but the effect was small (8% increase for each quintile increase in the Mediterranean diet score). Females, who as a group had greater overall adherence to the

Mediterranean diet compared to males, did not show an association between diet and sleep disturbance. One unexplored factor that may contribute to the discrepant results between males and females and the unexpected findings in males is the overall timing of meals and snacks. A previous study reported that compared to females, males tend to consume more calories in late-night hours (Spaeth et al., 2014), which may have a disruptive effect on sleep (Chung et al., 2020).

With respect to macronutrients, I found no significant associations between the intake of proteins, carbohydrates or fats and sleep disturbance. This is not consistent with a prior study that showed individuals with lower intake of each of these macronutrients experience shorter sleep duration compared to individuals with normal sleep duration (Grandner, M.A. et. al., 2013). Furthermore, diets high in protein have been associated with fewer awakenings, and diets high in carbohydrates have been associated with shorter sleep latency (i.e., falling asleep quicker; Lindseth et al., 2013). Of note, these studies utilized a more detailed scale in the assessment of sleep duration (Grandner, 2013; Lindseth et al., 2013), as compared with the present study that queried the presence or absence of sleep disturbance.

In the present study, there was a trend ($p = .059$) where increasing flavonoid intake was associated with a 1% reduction in the odds of sleep disturbance among females only. Flavonoids are phytonutrients found in foods such as tea, wine, leafy greens, onions, apples, berries, and cherries (Harborne & Williams, 2000; Liu, 2013). They are characterized as having strong antioxidant and anti-inflammatory properties (Kicinska & Jarmuszkiewicz, 2020) and have been associated with improved sleep quality in other studies (Godos et al., 2020; Fernandez et al., 2003; Jiang et al., 2007).

Flavonoid intake was not significantly associated with sleep disturbance among males, though it is noteworthy that intake was significantly lower in males (with less variability) compared to females. Intake thus, may have been insufficient in males to exert potential beneficial effects.

Interactions

Interactions between BDNF receptor-related SNPs and lifestyle factors yielded no significant associations with sleep disturbance in either males or females, except for a trend among females for Val66Met and physical activity. No studies have examined BDNF related SNPs and lifestyle factors on sleep disturbance. In the current study, significant results (p = .026) indicated that female carriers of the minor allele of val66MET, who engaged in lower levels of physical activity, had a 45% greater risk of sleep disturbances. As discussed above, there is evidence that BDNF levels affect sleep (Deuschle et al. (2018) and that physical exercise increases serum BDNF levels (Schmolesky et al., 2013). Thus, some studies support a relationship individually between serum BDNF levels and physical activity and BDNF levels and sleep. The interaction discussed above may suggest a moderating role of the val66MET genotype on the association between physical activity and sleep disturbance in females. Clearly, more research is needed to further explore these associations.

Strengths and Limitations

The current study had a number of strengths including a high participation rate (90% enrollment) from a large, population-based sample. The current study also examined four SNPs related to BDNF or its receptors and life-style factors while examining sex differences, all of which have been largely unexplored.

Limitations included the reliance of a single question of the presence/absence of sleep disturbance, which by its nature did not allow for an examination of underlying causes (e.g., obstructive sleep apnea or restless leg syndrome), which may be less associated with BDNF-related mechanisms for sleep. Also unexplored were other factors that may affect sleep such as medications or caffeine consumption. The study's population homogeneity—predominantly white, of Northern European heritage and predominant religious group of Latter-Day Saints —also hampers generalizability to culturally diverse groups or other racial groups. The latter may be relevant for BDNF-related SNPS as previous research has suggested population differences in minor allele frequencies [e.g., the proportion of Met allele carriers of the Val66Met BDNF gene is significantly higher in Asians than that in Caucasians (Chu et al., 2022)]. Additionally, other factors related to sleep were not examined including socioeconomic status nor time of day of physical activity or meals and snacks. Previous research has suggested that lower incomes are associated with shorter duration of sleep, higher body mass index, and consumption of food with lower nutrient contents (Grandner et. al., 2013). Additionally, as noted previously, time of day for physical activity or mealtimes may impact sleep (Yamanaka et al., 2015).

Future Directions

Given the multifactorial nature of sleep disturbances in older adults, it is recommended that future research adopt a comprehensive perspective. For future studies exploring the role of BDNF-related genes and sleep, measurements of circulating levels of BDNF in serum may be an important addition to explicate associations (or lack thereof) between sleep disturbance and SNPs for BDNF or its receptors, BDNF levels

and sleep disturbance as well as the role of diet and exercise in sleep duration and quality and their interactions with BDNF/receptor related SNPs. This dual focus may provide a more nuanced understanding of the mechanisms underlying associations in combination with lifestyle factors on sleep disturbances. Future researchers may wish to explore the role micronutrients such as vitamins and minerals have on sleep duration. Although the Mediterranean diet is rich with micronutrients (Castro-Quezada, I., et al., 2014), our study did not examine specific micronutrients or the use of dietary supplements. Previous studies have reported associations between inadequate intake of vitamins A, C, D, E, and zinc and short sleep duration (Ikonte et al., 2019; Grandner et al., 2013). Future research may also greatly benefit from incorporating physiological measures and objective measures of sleep such as sleep EEG which would allow one to examine different stages of sleep such as NREM and REM alongside a subjective measure of sleep quality such as the Pittsburgh Sleep Quality Index (Buysse et al., 1989). Doing so may offer the potential to capture characteristics of sleep quality with greater precision and depth. An exploration of sleep patterns such as differentiating between daytime napping and nocturnal sleep may also be relevant to the study of sleep quality and sleep disturbances. In the face of the significant numbers of older adults affected by insomnia (ranging between 30 - 50%; Peng et al., 202; Li et al., 2018), continued research in this field may identify modifiable factors and potential interventions to improve the quality of life for countless older adults and healthier aging for all.

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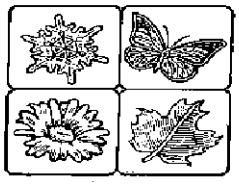
Appendix 1. PREDICTOR VARIABLES, COVARIATES, INTERACTIONS, AND OUTCOMES FOR EACH RESEARCH QUESTION

Research Question	Primary Predictors	Covariates	Interactions	Outcomes
1. Are SNPs that code for BDNF receptors associated with the occurrence of sleep disturbance?	rs6265 (BDNF Val66Met)	Age		Sleep disturbance (presence/absence)
	rs2289656 (BDNF receptor trkB)	BMI		
	rs2072446 (BDNF receptor $p75^{NTR}$)	Education		
	rs56164415 (BDNF C270T)	APOE ε4		
		Depression		
2. Are lifestyle factors of physical activity, diet pattern and macronutrients associated with sleep disturbance?	Physical Activity levels	Age		Sleep disturbance (presence/absence)
	Mediterranean Diet	BMI		
	Macronutrients (Carbohydrates, fats, proteins)	Education		
	Flavonoids	APOE ε4		
		Depression		
3. Do SNPs in Question 1 modify the associations between lifestyle factors in Question 2 and sleep disturbance?	Physical Activity levels	Age	SNP*Physical Activity	Sleep disturbance (presence/absence)
	Macronutrients (Carbohydrates, fats, proteins)	BMI	SNP*Macronutrients	
	Flavonoids	Education	SNP*Flavonoids	
	Mediterranean Diet	APOE ε4	SNP*Mediterranean Diet	
	rs6265 (BDNF Val66Met)	Depression		
	rs2289656 (BDNF receptor trkB)			
	rs2072446 (BDNF receptor $p75^{NTR}$)			
	rs56164415 (BDNF C270T)			

Appendix 2. CACHE COUNTY STUDY ON MEMORY IN AGING NUTRITION QUESTIONNAIRE



CACHE COUNTY STUDY ON MEMORY IN AGING
NUTRITION QUESTIONNAIRE
Conducted by: Utah State University

Marking Instructions

Please follow these few simple rules in completing this questionnaire.

1. Use only a pencil. (Please DO NOT use a pen)
2. Darken completely the circle of the answer you choose
3. Erase cleanly any answer that you wish to change
4. Make no stray marks of any kind on the form
5. For food that you never or rarely eat, please mark the first column labeled "None or Less than once a month. Please do not leave any food items blank.
6. Please note the correct way to mark the answers.

Correct Mark				Incorrect Mark			

Please answer the following. Check the appropriate gender, and fill in your height, weight, and age

Male_____ Female_____

Height_____ Weight_____ Age_____

THANK YOU!!!!

DIETARY SUPPLEMENTS

PLEASE INDICATE WHICH, IF ANY, OF THE FOLLOWING SUPPLEMENTS YOU ARE CURRENTLY TAKING. PLEASE ANSWER "YES" OR "NO" FOR ANY SUPPLEMENT LISTED.

1. Do you regularly take multivitamins
   □ NO> PLEASE GO TO QUESTION 2
   □YES> CONTINUE:
   (A) How many years have you taken multivitamins?
   □ 0-1 years □ 5-9 years
   □ 2-4 years □ 10 or more years
   (B) What specific brand do you use?_____

Excluding multivitamins, do you take any of the following supplements listed below?

2. Do you regularly take Vitamin A?
   □ NO> PLEASE GO TO QUESTION 3
   □ YES> CONTINUE:
   (A) How many years have you taken multivitamins?
   □ 0-1 years □ 5-9 years
   □ 2-4 years □ 10 or more years
   (B) What dose do you take per day?
   □ less than 8,000 IU □ 22,001 IU or more
   □8,001 to 13,000 IU □ Don't know
   □ 13,001 to 22,000 IU

3. Do you regularly take Vitamin C?
   □ NO> PLEASE GO TO QUESTION 4
   □YES> CONTINUE:
   (A) How many years have you taken multivitamins?
   □ 0-1 years □ 5-9 years
   □ 2-4 years □ 10 or more years
   (B) What dose do you take per day?
   □ less than 400 mg □ 1301 mg or more
   □ 401 to 700 mg □ Don't know
   □ 701 to 1300 mg

4. Do you regularly take Vitamin C?
   □ NO> PLEASE GO TO QUESTION 5
   □ YES> CONTINUE:
   (A) How many years have you taken multivitamins?
   □ 0-1 years □ 5-9 years
   □ 2-4 years □ 10 or more years
   (B) What dose do you take per day?
   □ less than 100IU □ 504 IU or more
   □ 101 to 300 IU□ Don't know
   □ 301 to 500 IU

5. Do you regularly take Calcium?
   □ NO> PLEASE GO TO QUESTION 6
   □ YES> CONTINUE:
   (A) How many years have you taken multivitamins?
   □ 0-1 years □ 5-9 years
   □ 2-4 years □ 10 or more years
   (B) What dose do you take per day?
   □ less than 400 mg □ 1301 mg or more
   □ 401 to 900 mg □ Don't know
   □ 901 to 1300 mg

6. Do you regularly take Vitamin D?
   □ NO> PLEASE GO TO QUESTION 7
   □ YES> CONTINUE:
   (A) How many years have you taken multivitamins?
   □ 0-1 years □ 5-9 years
   □ 2-4 years □ 10 or more years
   (B) What dose do you take per day?
   □ less than 200 IU □ 1,000 IU or more
   □ 201 to 400 IU □ Don't know
   □ 401 to 1,000 IU

7. Do you regularly take Vitamin B6?
   □ NO> PLEASE GO TO QUESTION 8
   □ YES> CONTINUE:
   (A) How many years have you taken multivitamins?
   □ 0-1 years □ 5-9 years
   □ 2-4 years □ 10 or more years
   (B) What dose do you take per day?
   □ less than 10 mg □ 80 mg or more
   □ 10 to 39 mg □ Don't know
   □ 40 to 79 mg

8. Do you regularly take Selenium?
   □ NO> PLEASE GO TO QUESTION 9
   □ YES> CONTINUE:
   (A) How many years have you taken multivitamins?
   □ 0-1 years □ 5-9 years
   □ 2-4 years □ 10 or more years
   (B) What dose do you take per day?
   □ less than 80 mcg □ 251 mcg or more
   □ 81 to 130 mcg □ Don't know
   □ 131 to 250 mcg

FOR OFFICE USE ONLY		
(A)	(B)	(C)
1 2 3 4 5 6 7 8 9 0	1 2 3 4 5 6 7 8 9 0	1 2 3 4 5 6 7 8 9 0
□ □ □ □ □ □ □ □ □ □	□ □ □ □ □ □ □ □ □ □	□ □ □ □ □ □ □ □ □ □
1 2 3 4 5 6 7 8 9 0	1 2 3 4 5 6 7 8 9 0	1 2 3 4 5 6 7 8 9 0
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9. Do you regularly take Iron?
□NO> PLEASE GO TO QUESTION 10
□YES> CONTINUE:
(A) How many years have you taken multivitamins?
□ 0-1 years □ 5-9 years
□ 2-4 years □ 10 or more years
(B) What dose do you take per day?
□ 50 mg or less □ 401 mg or more
□ 51 to 200 mg □ Don't know
□ 201 to 400 mg

10. Do you regularly take Zinc?
□ NO> PLEASE GO TO NEXT SECTION
□YES> CONTINUE:
(A) How many years have you taken multivitamins?
□ 0-1 years □ 5-9 years
□ 2-4 years □ 10 or more years
(B) What dose do you take per day?
□ less than 25 mg □ 101 mg or more
□ 26 to 75 mg □ Don't know
□ 76 to 100 mg

11. DO YOU TAKE ANY OF THE FOLLOWING OTHER SUPPLEMENTS:

Cod liver oil .....□ Yes .....□ No	Folic acid..... □ Yes .....□ No
Other fish oil .....□ Yes .....□ No	Iodine ..... □ Yes .....□ No
Niacin.....□ Yes .....□ No	Brewer's Yeast..... □ Yes .....□ No
Beta-caroten .....□ Yes .....□ No	Magnesium..... □ Yes .....□ No
Thiamine (vitamin B1).....□ Yes .... □ No	Any others? ..... □ Yes .....□ No
B-complex vitamins .....□ Yes .... □ No	If yes, please specify_____ _____

FOODS YOU EAT

For each food listed, please mark a circle for how often during the past year, on average, you have eaten the serving size specified. Be sure to mark a circle for every food listed. If you never eat the food listed mark the circle in the first column. **DAIRY FOODS**	**AVERAGE USE FOR PAST 12 MONTHS**								
	NONE OR LESS THAN 1 PER MO.	1-3 PER MO.	1 PER WK.	2-4 PER WK.	5-6 PER WK.	1 PER DAY	2-3 PER DAY	4-5 PER DAY	6 PER DAY
Skim or low fat milk (8 oz. glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Whole milk (8 oz. glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate milk or cocoa (8 oz. glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream or half-and-half, e.g. coffee, whipped (Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sour cream (Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Non-dairy coffee whitener (tsp)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sherbet, ice milk, or frozen yogurt (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ice cream (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Yogurt (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cottage or ricotta cheese (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream cheese (1 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other cheese, e.g. American, cheddar, etc., plain or as part of a dish (1 slice or 1 oz. serving)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Margarine (1 tsp, added to food or bread; exclude use in cooking	☐	☐	☐	☐	☐	☐	☐	☐	☐
Butter (1 tsp), added to food or bread; exclude use in cooking.	☐	☐	☐	☐	☐	☐	☐	☐	☐
FRUITS	☐	☐	☐	☐	☐	☐	☐	☐	☐
Raisins (1 oz. or small pack) or grapes (1/2 c)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Prunes (7 prunes or ½ cup)	□	□	□	□	□	□	□	□	□
Bananas (1)	□	□	□	□	□	□	□	□	□
Cantaloupe (1/4 melon)	□	□	□	□	□	□	□	□	□
Avocado (1/2 fruit or ½ cup)	□	□	□	□	□	□	□	□	□
Fresh apples or pears (1)	□	□	□	□	□	□	□	□	□
Apple juice or cider (small glass)	□	□	□	□	□	□	□	□	□
Oranges (1)	□	□	□	□	□	□	□	□	□
Orange Juice (small glass)	□	□	□	□	□	□	□	□	□
Grapefruit (1/2)	□	□	□	□	□	□	□	□	□
Grapefruit juice (small glass)	□	□	□	□	□	□	□	□	□
Other fruit juices (small glass)	□	□	□	□	□	□	□	□	□
Strawberries, fresh, frozen or canned (1/2 cup)	□	□	□	□	□	□	□	□	□
Blueberries, fresh, frozen or canned (1/2 cup)	□	□	□	□	□	□	□	□	□
Peaches, apricots or plums (1 fresh, or ½ cup canned)	□	□	□	□	□	□	□	□	□
VEGETABLES	□	□	□	□	□	□	□	□	□
Tomatoes (1)	□	□	□	□	□	□	□	□	□
Tomato juice, V8 (small glass)	□	□	□	□	□	□	□	□	□
Tomato sauce (1/2 cup) e.g. spaghetti sauce	□	□	□	□	□	□	□	□	□
Salsa or red chili sauce (1 Tbs)	□	□	□	□	□	□	□	□	□
Tofu or soybeans (3-4 oz.)	□	□	□	□	□	□	□	□	□
String (green) beans (1/2 cup)	□	□	□	□	□	□	□	□	□
Broccoli (1/2 cup)	□	□	□	□	□	□	□	□	□
Cabbage or cole slaw (1/2 cup)	□	□	□	□	□	□	□	□	□
Cauliflower (1/2 cup)	□	□	□	□	□	□	□	□	□
Brussels sprouts (1/2 cup)	□	□	□	□	□	□	□	□	□
Carrots, raw (1/2 carrot or 4 sticks)	□	□	□	□	□	□	□	□	□
Carrots, cooked (1/2 cup) or carrot juice (2-3 oz.)	□	□	□	□	□	□	□	□	□
Red Beets—not greens (1/2 cup)	□	□	□	□	□	□	□	□	□
Corn (1 ear or ½ cup frozen or canned)	□	□	□	□	□	□	□	□	
Peas or lima beans (1/2 cup fresh, frozen, or canned)	□	□	□	□	□	□	□	□	□
Mixed vegetables (1/2 cup)	□	□	□	□	□	□	□	□	□
Beans or lentils, baked or dried (1/2 cup)	□	□	□	□	□	□	□	□	□

Dark orange (winter) squash (1/2 cup) (acorn, butternut squash)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Eggplant, zucchini or other summer squash (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Yams or sweet potatoes (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spinach, cooked (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spinach, raw as in a salad (1 cup serving)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kale, mustard or chard greens (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Iceberg or head lettuce (1 cup serving)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Romaine or leaf lettuce (1 cup serving)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Celery (2-4 4" sticks)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweet green or red peppers (3 slices or ¼ pepper)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Onions as a garnish, or in salad (1 slice)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Onions as a vegetable, rings or in soup (1 onion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
EGGS, MEATS, ETC.									☐
Eggs (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken with skin (4-6 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken without the skin (4-6 oz.), includes grilled chicken sandwich	☐	☐	☐	☐	☐	☐	☐	☐	☐
Turkey, including ground turkey (4-6 oz. or 2 turkey dogs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hot dogs 91)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bacon (2 slices)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Processed meats, e.g. sausage, salami, bologna, etc. (1 piece or slice)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hamburger (1 patty)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Taco or tostado (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Burrito (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Enchilada (2)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef, pork or lamb as a sandwich or mixed dish, e.g. stew, casserole, lasagna, chili etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pork as a main dish, e.g. ham or chops (4-6 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef or lamb as a main dish, e.g. steak, roast (4-6 oz)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Liver: beef, calf, or pork (4 oz)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Liver: chicken or turkey (2 oz)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Canned tuna fish (3-4 oz)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dark meat fish, e.g. mackerel, salmon, sardines, bluefish, swordfish (3-5 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fried fish, e.g. fish sticks, fish and chips style fish (3-5 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other fish (3-5 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Shrimp, lobster, scallops as a main dish	☐	☐	☐	☐	☐	☐	☐	☐	☐

BREADS, CEREALS, STARCHES									☐
Cold breakfast cereal (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cooked oatmeal/cooked oat bran (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other cooked breakfast cereal (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Instant breakfast beverage, e.g. Carnation	☐	☐	☐	☐	☐	☐	☐	☐	☐
White bread (slice), including pita bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dark bread (slice), including pita bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
English muffins, bagels, or dinner rolls (1 each)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Muffins or biscuits (1 each)	☐	☐	☐	☐	☐	☐	☐	☐	☐
White rice (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pasta, e.g. spaghetti, noodles, etc (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tortillas (1-10 inch shell)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other grains, e.g. bulgur, kasha, couscous, etc (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pancakes or waffles (2 each)	☐	☐	☐	☐	☐	☐	☐	☐	☐
French fried potatoes (4 oz. or size of small fries order)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Potatoes, baked, boiled (1each), or mashed (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Potato chips or corn chips (small bag or 1 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Crackers, e.g. Triscuits, Wheat Things (5 each)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pizza (2 slices)	☐	☐	☐	☐	☐	☐	☐	☐	☐
BEVERAGES									☐
Plain water, bottled or tap (1 cup or 8 oz. glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hawaiian Punch, lemonade, or other non-carbonated fruit drinks (1 glass, bottle, can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low-calorie cola, e.g. Diet Coke with caffeine (can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low-calorie caffeine-free cola (can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other low-calorie carbonated beverage, e.g. Fresca, Diet 7-UP (can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
7-Up, diet ginger ale (can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coke, Pepsi, or other cola with sugar (can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caffeine Free Coke, Pepsi, or other cola with sugar (can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other carbonated beverages with sugar, e.g. Sprite, Root beer (can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Regular Beer (1 glass, bottle, or can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Light Beer (1 glass, bottle, or can)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Red wine (4 oz. glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
White Wine (4 oz. glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Liquor, e.g. whiskey, gin, etc (1 drink or shot)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dark tea with caffeine (1 cup), not herbal tea	☐	☐	☐	☐	☐	☐	☐	☐	☐
Green tea or herbal tea (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coffee with caffeine (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Decaffeinated coffee (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
SWEETS, BAKED BGOODS, MISC									☐
Chocolate (bar or packet) e.g. Hershey's M & M's	☐	☐	☐	☐	☐	☐	☐	☐	☐
Candy bars, e.g. Snicker, milky way, Reeses	☐	☐	☐	☐	☐	☐	☐	☐	☐
Candy other than chocolate (1 oz)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cookies, home baked (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cookies, ready make (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brownies (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Doughnuts (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cake, home baked (1 slice)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cake, ready make (1 slice)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pie, homemade (1 slice)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pie, ready made (1 slice)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweet roll, coffee cake or other pastry, home baked (1 each)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweet roll, coffee cake or other pastry, ready made (1 each)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Jams, jellies, preserves, syrup, or honey (1 Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peanut butter (1 Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Popcorn (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peanuts (small packet or 1 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other nuts (small packet or 1 oz.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Oat bran, added to food (1 Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other bran, added to food (1Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wheat germ (1 Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chowder or cream soup (1 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Olive oil salad dressing (1 Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other oil and vinegar dressing, e. g. Italian (1 Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mayonnaise or other creamy salad dressing (1 Tbs)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salt added at table (1 shake)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Garlic (1 clove or 4 shakes)	☐	☐	☐	☐	☐	☐	☐	☐	☐

FOOD PREPARATION	
1.	Do you eat cold breakfast cereal? ☐ NO> PLEASE GO TO NEXT QUESTION ☐ YES> What kind do you usually eat?_____

2.	How many teaspoons of sugar do you add to your beverages or food each day? □ 0-1 □ 2-4 □ 5-9 □10 or more
3.	When you have beef or lamb as a main dish, how is the meat cooked? □ Rare □ medium □ well □ Medium rare□ medium well □ do not eat meat
4.	How much of the visible fat on your beef, pork, or lamb do you remove before eating? □ remove all visible fat □ remove none □ remove most visible fat □do not eat meat □ remove small part of visible fat
5.	How often do you eat food that is fried at home? (exclude Pam-type spray) □ less than once per week □ 4-6 times per week □ 1-3 times per week □ daily
6.	How often do you eat fried food away from home? (e.g. french fries, fried chicken, fried fish). □ less than once per week □ 4-6 times per week □ 1-3 times per week □ daily
7.	What type and brand of cooking oil or fat do you usually use at home (e.g. corn oil, Mazola brand; lard) Type:_____ Brand:_____
8.	How does the amount of food you eat now compare to the amount you ate five years ago? □ I eat almost the same □ I eat less now □ I eat more now
9.	What was the main source of your drinking water over the past year? □ city system □ rural or county system □ private well □ bottled water □ other (please specify_____)

FOR OFFICE USE ONLY		
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YOUR ACTIVITES

1. About how many hours per day do you spend in light activity, such as walking, shopping, child care, cooking, carrying light objects, cleaning, and repairing?

   Hours per day_____

2. About how often do you take part in moderate physical activities including bowling, golf,

light swimming, gardening, walks over 15 minutes, fishing, light bicycling, or other light sports.

□Usually every day
□ 2-6 times a week
□ About once a week
□ A few times a month
□ A few times a year
□ Rarely or never

3. About how often do you take part in vigorous physical activity including jobbing, tennis, racquetball or squash, lap swimming, aerobics, vigorous bicycling, skiing, hiking, hunting or other vigorous sports…
   - □Usually every day
   - □ 2-6 times a week
   - □ About once a week
   - □ A few times a month
   - □ A few times a year
   - □ Rarely or never

4. How often do you talk on the telephone with family, friends, or neighbors?
   - □Usually every day
   - □ 2-6 times a week
   - □ About once a week
   - □ A few times a month
   - □ A few times a year
   - □ Rarely or never

5. How often do you get together with family, friends, or neighbors? This includes meeting in your own home, meeting in other's homes, or going out together.
   - □Usually every day
   - □ 2-6 times a week
   - □ About once a week
   - □ A few times a month
   - □ A few times a year
   - □ Rarely or never

6. How often do you attend meetings of social clubs, groups, or organizations such as bridge clubs, book clubs, hospital volunteer, gardening clubs, Rotary club, Kiwanis, VFW, etc.
   - □Usually every day
   - □ 2-6 times a week
   - □ About once a week
   - □ A few times a month
   - □ A few times a year
   - □ Rarely or never

Thank you for completing this questionnaire. Please make sure that no questions or pages have been skipped. Please place it in the postage-paid envelope that has been provided and seal it. Please return it to us in the mail.

Thank you for your time and cooperation. You have made an important contribution to our study of nutrition and health.

Utah State University