DISSERTATION

NEURODEGENERATIVE DISEASE AND OCCUPATIONAL MAGNETIC FIELD EXPOSURE: CASE CONTROL INVESTIGATIONS AND A BIOLOGICAL MARKER STUDY USING THE AMYLOID-BETA PROTEIN

Submitted by

Curtis W. Noonan, M.A.

Department of Environmental Health

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring, 2000

COLORADO STATE UNIVERSITY

December 10, 1999

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY CURTIS W. NOONAN ENTITLED "NEURODEGENERATIVE DISEASE AND OCCUPATIONAL MAGNETIC FIELD EXPOSURE: CASE CONTROL INVESTIGATIONS AND A BIOLOGICAL MARKER STUDY USING THE AMYLOID-BETA PROTEIN" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Thomas J. Keefe, Ph.D.

Howard S. Ramsdell, Ph.D.

Daniel H. Gould, D.V.M., Ph.D.

Kathy Magnusson, D.V.M., Ph.D.

Advisor – John S. Reif, D.V.M., M.Sc.

Department Head

ABSTRACT OF DISSERTATION

NEURODEGENERATIVE DISEASE AND OCCUPATIONAL MAGNETIC FIELD EXPOSURE: CASE CONTROL INVESTIGATIONS AND A BIOLOGICAL MARKER STUDY USING THE AMYLOID-BETA PROTEIN

Two studies were conducted to investigate recent epidemiological findings of an association between occupational exposure to magnetic fields and neurodegenerative diseases. The objective of the first study was to determine whether individuals whose death certificate indicated Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, or brain cancer were more likely to have had occupations associated with magnetic field exposure. Three methods of exposure assessment were applied to each case-control group. Amyotrophic lateral sclerosis and Parkinson's disease were associated with history of electrical occupations: odds ratio (OR) and 95% confidence interval (CI) = 2.30 (1.29, 4.09) and 1.55 (0.98, 1.76), respectively. A weak association was found for Alzheimer's disease [OR and 95% CI = 1.21 (0.83, 1.76)] using job title and industry to assess exposure. A weak association was found for brain cancer using a job exposure matrix [OR and 95% CI = 1.32 (0.92, 1.89). Our findings suggest that associations between occupational exposure to magnetic fields and risk of neurological diseases are sensitive to methods of exposure assessment.

The objective of the second study was to assess the relationship between occupational magnetic field exposure, melatonin, and concentrations of blood-borne soluble amyloid beta (Abeta), a protein associated with the pathological lesions of Alzheimer's disease. Blood and urine samples were obtained from male electric utility workers (n = 60) to quantify two lengths of the protein, Abeta(1-40) and Abeta(1-42), and the melatonin metabolite, 6-hydroxymelatonin sulfate (6-OHMS). Magnetic field exposure was assessed using personal data logging devices. There was no association between measures of magnetic field intensity and Abeta, but there was an inverse association between magnetic field variability and Abeta(1-42) and the ratio of Abeta(1-42) to Abeta(1-40). It was unclear whether variability was a magnetic field characteristic required to perturb Abeta concentrations or whether this exposure was a surrogate for other important factors such as physical activity. There was also suggestion of an inverse association between Abeta and post-workshift 6-OHMS.

Overall, these two studies offer some support for an association between occupational magnetic field exposure and neurodegenerative disease. The potential for future investigations to more fully elucidate this relationship are discussed.

Curtis William Noonan, M.A.
Department of Environmental Health
Colorado State University
Fort Collins, CO 80523
Spring 2000

ACKNOWLEDGMENTS

I gratefully acknowledge the participation of employees from Platte River Power Authority, Poudre Valley Rural Electric Authority, and the City of Fort Collins Division of Light and Power. The research project was funded through a grant from National Institute of Environmental Health Sciences and the blood draws were funded through a grant from the Platte River Power Authority. I also received financial support from an American Public Power Association scholarship sponsored through the Platte River Power Authority.

I am indebted to numerous individuals for their advice and support during this research project. I thank Dr. John S. Reif for his mentorship, friendship, tennis instruction, and invaluable assistance throughout my tenure as a student and researcher at Colorado State University. At various stages of this research project, I received advice from my Committee: Dr. Thomas J. Keefe, Dr. Howard S. Ramsdell, Dr. Daniel H. Gould, and Dr. Kathy Magnusson. I am particularly thankful for the support of Dr. James B. Burch without whose previous work this project would not have been possible. The contributions of research assistants Travers Ichinose and Terri Koleber were essential to the collection of data and timely completion of the study. Ken Webster provided computer programming assistance. The blood samples were collected by Shirley Mayfield, R.N. I also acknowledge the contribution of Carol Garrett and Katy

Meng of the Colorado Department of Public Health and Environment for acquiring the death certificate records for the case-control studies. The job exposure matrix used in the case-control studies was developed by Dr. Michael Yost and Jennifer Touchstone of the University of Washington's Department of Environmental Health.

For the Abeta analysis, I received training from Dr. Pankaj Mehta and Sangita Mehta from the Department of Immunology, New York State Institute for Basic Research, Staten Island, NY. They also provided me with antibodies, standards, and continued support with materials and methods. I am particularly grateful to Dr. Kathy Magnusson of the Colorado State University Department of Anatomy and Neurobiology for the use of her laboratory and Ginger Sammonds for her flexibility in making the laboratory available. Urinary 6-OHMS assays were performed by Ms. Xiaoming Sha under the supervision of Dr. Terry Nett, Director of the Radioimmunoassay Laboratory for the Animal Research and Biotechnology Laboratories. Creatinine assays were performed by Travers Ichinose in the laboratory of Dr. Martin Fettman of the Department of Pathology.

Equally important to the scientific contribution of those mentioned above was the emotional support provided to me by my family and friends. I would particularly like to acknowledge the love and support of my wife, Theresa Noonan, and my son, Jack Noonan, who endured many long nights and weekends in my absence. My father, Richard E. Noonan, also was a constant source of encouragement and support.

DEDICATION

This research is dedicated to the memory of my mother, Zara Gay Noonan, who inspired me to pursue my dreams and whose insightful words, "no one ever died from lack of sleep," helped get me through many deadlines.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

The incidence of neurodegenerative diseases such as Alzheimer's disease and amyotrophic sclerosis is much higher among older populations. While early onset Alzheimer's disease does occur in approximately 5% of cases, the vast majority of cases develop clinical symptoms after the age of 60.[1] Statistics on the prevalence and incidence of Alzheimer's disease are subject to a great deal of variability. Studies using routine health care facility data have considerably lower prevalence estimates than community-based surveillance data. Two studies utilizing the latter method reported prevalence estimates of approximately 10% for persons over the age of 65.[1] Because the disease is age-associated the incidence of the disease increases precipitously with age. Estimates of annual incidence rates range from 0.6% for persons 65-69 years old to 8.4% for persons over 85 years old.[1]

Compared with Alzheimer's disease, the age distribution of amyotrophic lateral sclerosis (ALS) is younger, yet the incidence rate increases steeply with age, and the median age at diagnosis is over age 60.[2,3] A much less common condition than Alzheimer's disease, ALS is estimated to have an incidence in the United States range from 1.4 to 1.8 per 100,000 people/year.[3,4] Given that the median duration of survival with ALS is slightly over 2 years as compared to as many as 20 years for patients

diagnosed with Alzheimer's disease, the prevalence of ALS is also considerably lower at 6 per 100,000 people.[3,4]

As in many other industrial countries, the elderly population of the United States is the fastest growing age group. The number of people over the age of 65 is expected to increase from 31.6 million in 1990 to 68.1 million in 2040.[5] Based on a United States Census Bureau population growth model, the estimated annual mortality from neurodegenerative diseases will increase 179% between 1990 and 2040.[6] In absolute terms, the number of deaths due to neurodegenerative disease will equal deaths due to female breast cancer projected for 2040, approximately 80,000.[5]

In addition to being debilitating and fatal diseases, many neurodegenerative disorders are also extremely costly as patients become dependent upon long term care. Nursing home care for an Alzheimer's patient can range from $42,000 to $75,000 per year, and the average lifetime cost per patient is $174,000.[7] The public health impact of ALS is less severe. The health care cost is much lower than Alzheimer's disease due to the shorter survival for the disease, but the lower age distribution of the disease suggests that ALS accounts for some productive years of life lost. In either case, most of the early pathogenic factors of these two neurodegenerative diseases are unknown, and successful preventative or curative therapies are not available.

The possible association between exposure to magnetic fields and chronic disease outcomes has been studied for nearly two decades. Although there is some degree of heterogeneity among the results, a pooling of studies on occupational exposure to magnetic fields and cancers of the central nervous system have demonstrated a small but significantly elevated risk.[8] More recently, a number of investigations have explored the

potential relationship between magnetic fields and other diseases of the central nervous system. Specifically, individuals employed in occupations considered to have medium to high exposure to magnetic fields have been shown to be at increased risk for Alzheimer's disease and ALS.[9-18] These neurodegenerative disorders are considered to be complex diseases, perhaps involving a combination of genetic and environmental factors. The establishment of magnetic fields as a contributory factor in the development of these diseases could have far reaching implications in terms of regulation, treatment, and an understanding of neurodegenerative etiology.

1.2 Purpose of the research

This project included two separate studies to explore the relationship between neurodegenerative disease and occupational exposure to magnetic fields. The first study was a series of death certificate-based case-control investigations to test the following hypothesis:

> Individuals whose death certificate indicates Alzheimer's disease or amyotrophic lateral sclerosis as an underlying, contributory, or primary cause of death are more likely to have had occupations with higher exposure to magnetic fields than individuals without these diseases.

To answer this question, we conducted four case-control studies using death certificate data in the state of Colorado. In addition to the Alzheimer's disease and ALS case-control groups, brain cancer and Parkinson's disease were used as positive and

negative comparison analyses, respectively, to validate the study design. Three different methods were used to assess magnetic field exposure based on the job titles listed on the records. We explored the following specific objectives:

1. Are individuals with Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, or brain cancer as an underlying, contributing, or primary cause of death more likely to have had electrical occupations than individuals with no indication of these diseases listed on their death certificate?

2. Are individuals with Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, or brain cancer as an underlying, contributing, or primary cause of death more likely to have had occupations considered to have definite or probable magnetic field exposure than individuals with no indication of these diseases listed on their death certificate?

3. Are individuals with Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, or brain cancer as an underlying, contributing, or primary cause of death more likely to have had occupations with a higher estimated time weighted average magnetic field exposure than individuals with no indication of these diseases listed on their death certificate?

4. Are there differences in the results obtained dependent upon the method used to assess occupational magnetic field exposure?

The second study examined the effect of magnetic field exposures on a biomarker related to Alzheimer's disease. This study of electric utility employees was designed to test the following hypothesis:

> Occupational exposure to magnetic fields results in higher circulating concentrations of amyloid beta (Abeta), a protein associated with the pathological lesions observed in Alzheimer's disease.

To address this hypothesis we assessed magnetic field exposures among electric utility employees using real-time monitoring devices and obtained a blood sample from each participant to assess concentrations of Abeta in plasma. We also collected data on descriptive characteristics and other exposure variables that may influence Abeta processing, as well as urinary concentrations of the melatonin metabolite, 6-hydroxymelatonin sulfate (6-OHMS). We explored the following specific objectives:

1. Are there increased concentrations of circulating Abeta among persons occupationally exposed to magnetic fields? Magnetic field exposure metrics include measures of field intensity and temporal stability. Plasma concentrations of two lengths of Abeta, Abeta(1-40) and

Abeta(1-42), as well as the ratio between the two, were used as dependent variables.

2. Are low urinary concentrations of the melatonin metabolite, 6-OHMS, associated with high concentrations of circulating Abeta?

3. What other individual descriptive or exposure variables are associated with concentrations of circulating Abeta?

1.3 Organization of the Dissertation

A literature review covering topics related to both of the studies is presented in Chapter Two. The first section of the literature review provides a background for the death certificate study and includes descriptions of previous epidemiological investigations of neurological diseases and occupational exposure to magnetic fields. The next two sections of the literature review relate specifically to the current study of electric utility workers. There is a discussion of the relevance of using blood-borne soluble Abeta as a marker in this study, followed by a review of the potential mechanisms whereby magnetic fields and melatonin could be related to the expression of Abeta. Descriptions and results of the two studies are presented in manuscript format in Chapters Three and Four. As such, there is some repetition of the materials covered in the literature review. Figures and tables that are to be included in the manuscripts are attached at the end of their respective chapters. In order to include items that would be considered superfluous for a publishable manuscript, yet were essential in the

methodology or analysis phases of this research project, appendices are attached at the end of the dissertation. Where appropriate, references to tables, figures, and descriptions contained within the appendices are noted in the respective manuscripts. As discussion and conclusions for each study are presented within the respective manuscripts, Chapter Five is a brief presentation of overall conclusions and directions for future research.

CHAPTER 2

LITERATURE REVIEW

This literature review is divided into three sections and will cover subject matter for both of the following studies. The first section describes the genetic and environmental factors associated with neurodegenerative diseases with particular emphasis on occupational exposure to magnetic fields as a risk factor. A brief discussion of brain cancer is also included since this disease was used as a comparison analysis in the death certificate study. The second section provides a rationale for using circulating levels of the amyloid beta protein (Abeta) as a marker for the future presentation of Alzheimer's disease. The final section outlines the potential relationships between magnetic field-induced biological effects and the processing of Abeta with particular emphasis on the role of melatonin.

2.1 Epidemiology of neurological diseases and magnetic field exposure

Genetic linkages have been identified for many neurodegenerative diseases, yet heredity cannot fully explain the prevalence of these conditions. Environmental exposures may play a role in neurodegenerative disease etiology in combination with, or separate from, genetic susceptibilities. Recent epidemiological studies have suggested an association between occupational exposure to magnetic fields and neurodegenerative

diseases, specifically Alzheimer's disease and ALS. Below is a review of the genetics involved in these diseases and the relevant epidemiological studies for these endpoints as well as for brain cancer and Parkinson's disease.

2.1.1 Alzheimer's disease

The neuritic plaques and neurofibrillary tangles associated with the most common form of age-associated dementia were first described by Alois Alzheimer in 1907.[19] Neurological damage in Alzheimer's disease is most pronounced in the hippocampus and parts of the neocortex. Neuronal loss in these brain regions appears to affect the cholinergic, glutaminergic, noradrenergic, and serotonergic systems.[19] Clinical symptoms include progressive decline in multiple cognitive areas, including memory, aphasia, apraxia, and agnosia. Standardized diagnostic criteria have yielded accuracy rates of 85% or greater, but the post-mortem observation of the neuropathological lesions remains the only definitive diagnosis for Alzheimer's disease.[20]

Some of the genetic linkages in Alzheimer's disease have been well established. A number of point mutations on the chromosome 21 gene that codes for the amyloid beta precursor protein (APP) have been associated with early onset familial cases.[21] Mutations on chromosomes 14 and 1 also have been present in some cases of familial Alzheimer's disease.[22] The mutations on these three chromosomes are extremely rare and account for only a small proportion of cases. A more significant genetic variation with respect to sporadic Alzheimer's disease occurs on chromosome 19, specifically the gene that codes for apolipoprotein-E (APOE).[23] APOE is a blood protein that transports cholesterol and can occur as APOE2, APOE3, or APOE4. Homozygosity and

heterozygosity for the APOE4 allele is associated with a tenfold and a fourfold increase in risk of Alzheimer's disease, respectively. Moreover, approximately 31% of the population carry at least one allele for APOE4.[23]

These genetic discoveries, however, are still limited in fully explaining the etiology and age of onset of Alzheimer's disease. Four large, population-based twin studies reinforced the genetic contribution to Alzheimer's disease etiology but also suggested the importance of environmental influences.[24-27] A Swedish twin study reported 67% and 22% concordance rates for monozygotic and dizygotic twins, repectively.[24] The higher concordance rate among monozygotic twins was also observed in other twin studies, confirming the influence of genetic factors.[25-27] Two studies, using tetrachoric correlations and structural modeling to estimate genetic and environmental components of variance, suggested a simple heritability of 0.74 to 0.80.[24,28] Thus, based on twin studies, environmental factors may account for 20 to 25% of the variance in population vulnerability to Alzheimer's disease. Differences in age of onset among twin pairs also suggested an environmental role in Alzheimer's disease etiology. In the Swedish study among twins concordant for Alzheimer's disease, only 50% had an age at onset within five years of one another.[24]

Several epidemiological investigations have attempted to identify environmental risk factors for Alzheimer's disease. Head trauma, low levels of education, and aluminum exposure are among the risk factors that were inconsistently associated with Alzheimer's disease.[29] Recent occupational studies have suggested a relationship between occupational exposure to magnetic fields and Alzheimer's disease.[9-11,13,14,17] Sobel et al.[9,10] analyzed four independent populations of Alzheimer's disease cases and

controls. The authors found an association between occupations having medium to high magnetic field exposure and Alzheimer's disease with relative risk estimates across all the studies ranging from 2.9 to 3.9. When three of the data sets were combined, the association was statistically significant for females and both genders combined (odds ratios (OR) and 95% confidence intervals (CI) = 3.9 (1.7 – 8.9) and 2.9 (1.6 – 5.4), respectively). The combined analysis for men alone indicated an elevated, yet not statistically significant, odds ratio for Alzheimer's disease associated with magnetic field exposure. Exposure classification in these case-control studies was based upon primary lifetime occupation as determined by surrogate interview for cases and demented controls and direct interview for non-demented controls. The use of a dichotomous exposure variable and the lack of information on duration of employment prevented the authors from establishing a cumulative dose-response relationship. A similar study in Sweden with more quantitative exposure assessment found a higher risk for Alzheimer's disease among subjects enrolled in a twin study whose most recent job had an average magnetic field exposure of more than 2.0 milliGauss (mG).[17] The estimated relative risk for Alzheimer's disease was 2.4 and 2.7 against two separate control groups, and when vascular dementia cases were combined with Alzheimer's disease cases, the OR (and 95% CI) rose to 3.3 (1.3 – 8.6) and 3.8 (1.4 – 10.2).[17] This result is interesting given the fact that the clinical manifestations of vascular dementia are often indistinguishable from Alzheimer's disease, and autopsy studies have shown that patients diagnosed with vascular dementia often have the neuropathological lesions associated with Alzheimer's disease.[30,31]

Investigations of occupational magnetic field exposure and Alzheimer's disease with mortality as the endpoint have offered mixed results. A study by the National Institute for Occupational Safety and Health (NIOSH) suggested an association between occupations with probable magnetic field exposure and Alzheimer's disease mortality.[11] NIOSH assessed proportionate mortality ratios (PMRs) by occupation from death certificates in over 27 states from 1982-1991. Although not hypothesized *a priori*, the study found an occupational clustering of electrical workers with Alzheimer's disease as an underlying or contributory cause of death. Specifically, they found elevated PMRs for electricians, power transmitter installers, electrical and electronic technicians, broadcast operators, and electrical and electronic engineers.[12] In addition to problems of exposure misclassification, this study did not take into account the potential for heterogeneous reporting of neurological disease among the states. Another death certificate-based study utilizing a similar data set reported a slight, statistically significant risk for Alzheimer's disease among individuals with electrical occupations (OR and 95% CI = 1.2 (1.0 – 1.4)).[13] Finally, a review of Alzheimer's disease deaths among a large cohort of electric utility workers demonstrated a modest, yet imprecise, rate ratio (RR) of 2.1 (95% CI = 0.6 – 6.8) for individuals with magnetic field-exposed jobs for more than 20 years.[14] There was also suggestion of a dose-response relationship when looking at strata of cumulative career exposure, yet none of the individual strata achieved statistical significance.[14] These findings were observed when cases were selected based on underlying cause of death, but RRs were close to unity when using any mention of Alzheimer's disease as the selection criterion.[14] The above studies suggest there may be a modest association between occupational magnetic field exposure and risk of

developing Alzheimer's disease, yet further investigations are required to corroborate these findings.

2.1.2 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is the most common form of the motor neuron diseases, a general term used to describe diseases of the anterior horn cells and motor system. Axonopathy, or "dying back" degeneration, in ALS affects lower motor neurons, upper motor neurons, or a combination of the two.[32] The disease often begins asymmetrically and distally in one limb and spreads to involve contiguous motor neurons. Early clinical symptoms include fatigue and weakness in at least one limb, but sensory and autonomic functions are seldom affected.[33] Life expectancy is generally two to five years, but a small proportion of ALS patients can survive for several years.[34]

While some genetic factors have been associated with ALS, it is believed to be a neurodegenerative disease of multifactorial etiology. This disease can be classified into three major groups: familial, sporadic, and the ALS-Parkinson's dementia complex (ALS/PDC) found in geographically isolated areas. Autosomal-dominant familial (FALS) forms of the disease account for less than 10% of all cases. Linkage studies of FALS have identified several point mutations on the gene coding for Cu,Zn superoxide dismutase, yet only 10 to 20% of all familial cases could be attributed to the superoxide dismutase gene.[35] Due to the relative low incidence of the disease, there have not been large population-based twin studies of ALS. Case reports, however, cited a pair of monozygotic twins separated in early childhood that were discordant for ALS and a pair of dizygotic twins concordant for the disease.[36,37] Little can be concluded from such

reports, yet these cases and the relatively low proportion of directly inheritable cases suggest non-genetic factors are important in the etiology of ALS. Observations of ALS/PDC in the high risk foci, although clinically distinct from classical ALS, also indicate the involvement of environmental factors. Non-Chamorro immigrants to Guam had experienced ALS/PDC incidence rates similar to that observed among the native population.[34] Moreover. the high incidence of ALS/PDC observed in the high risk foci has steadily declined since the 1960s, suggesting a role for contact with environmental factors or human activities that have changed over time.[34] Potential environmental factors that have been studied in these high risk foci include exposure to certain metals and consumption of a native seed which contains a neurotoxic amino acid.[38,39]

In addition to descriptive characteristics of the disease, such as its association with age and a male to female ratio of approximately 1.4:1, epidemiological investigations of sporadic ALS have explored a number of potential risk factors. Some reports have found that a history of physical trauma, including bone fractures and surgical operations, was more frequent among ALS cases compared to controls,[16,40-42] but this was not consistently observed in all studies.[3,43-45] History of electrical shock has been reported as a specific trauma associated with ALS. A study conducted in the United Kingdom observed 15 cases versus 5 controls who had been exposed to either electrical shock or lightning strike, but the authors did not specify the severity of the electrical shock events.[42] Another investigation, reporting on electric shock events resulting in unconsciousness, found a statistically significant OR of 2.8 (95% CI = 1.0 – 9.9).[16] In contrast to these observations, two studies in Japan found similar frequencies of electrical shock among ALS cases and controls.[40]

Several reports exploring potential risk factors for ALS have reported an association with occupations that are often used to classify individuals with probable exposure to magnetic fields. One of the groups reporting electric shock events as a risk factor also observed an odds ratio of 3.8 (95% CI = 1.4 – 13.0) for individuals with electrically-related occupations.[16] While the use of this occupational grouping was included to further substantiate the observed association with antecedent electrical shock events, the vast majority of the selected job titles were the same ones often used to describe individuals with likely exposure to magnetic fields. An occupational case-control study in Sweden also found elevated, yet imprecise, relative risk estimates for occupations that would be expected to have higher exposure to magnetic fields.[46] Specifically, electricity workers had an odds ratio of 1.5 (95% CI = 0.9 - 2.6), and communications workers had an odds ratio of 6.7 (95% CI = 0.8 - 57.3).[46] Two studies found welders, an occupation with high magnetic field exposure, to have elevated risk for ALS,[46,47] yet this occupation is also associated with exposure to neurotoxic metals. The NIOSH death certificate study, mentioned above, found elevated PMRs for ALS among power plant operators, electrical and electronic equipment repairers, electrical engineers, and airline pilots and navigators.[11,12] A review of World War II veterans with ALS listed on their death certificate showed excess cases among individuals with a pre-service occupation in the general category of Operatives.[41] Most of this excess was attributed to a group labeled 'operative not elsewhere classified' which included drill press operators, lathe operators, and mill hands.[41] Only weak inferences can be made with regard to magnetic field exposures within these job categories, and these pre-service occupations may have been short relative to lifetime exposure. Another study exploring causes of

death among civilian pilots and navigators, occupations with relatively high exposures to magnetic fields, found a significantly elevated PMR of 2.4 (95% CI = 1.0 – 4.6) for ALS deaths.[48] Finally, although case reports offer little in the way of establishing an etiological cause and effect relationship, one individual developed ALS after working for several years in a clothing plant with high magnetic field readings.[49] The individual's work station ranged from 25 to 75 mG with the highest readings occurring where he rested his feet, the portion of the body accounting for the patient's first symptoms.[49]

Recent studies, specifically looking at lifetime occupational magnetic field exposures as an *a priori* risk factor, found an increased risk for ALS.[13-15,18] In a clinic based study of ALS patients, the ORs (and 95% CIs) were 7.5 (1.4 – 38.1) and 5.5 (1.3 – 22.5) among the upper quartiles of magnetic field exposure for total occupational exposure and average occupational exposure, respectively.[15] Savitz et al.[14] reviewed occupational data from five electric utilities and found a significant association between magnetic fields and ALS deaths, particularly when looking at exposures of long duration (OR and 95% CI = 3.1 (1.0 – 9.8)). In contrast to the earlier ALS studies discussed above, both of these recent studies utilized magnetic field exposure assessments specific to job title and duration of employment. A review of ALS deaths among Danish utility workers demonstrated an increasing trend in standardized mortality ratios (SMRs) by quartile of estimated magnetic field exposure.[18] Due to the low number of cases, these findings were not statistically significant, but combining the upper two quartiles yielded an SMR of 2.5 (95% CI 1.1 – 4.8).[18] A mortality study utilizing death certificates from 25 states found a modest risk for ALS deaths among males within the common magnetic field-related occupational grouping of electrical workers (OR and 95% CI = 1.5 (1.1 –

1.6).[13] Risk estimates were elevated across most specific occupations within this grouping for which there were sufficient numbers of cases.

2.1.3 Parkinson's disease

Parkinson's disease is characterized by the degeneration of dopamine-producing neurons within the substantia nigra. This condition is age-associated, and presentation of the disease does occur until approximately 80% of the dopaminergic neuronal population is lost. The resultant reduction in the levels of this inhibitory neurotransmitter can cause tremor, rigidity, and akinesia. Exogenous administration of levodopa can alleviate these symptoms, but eventually the patient fails to respond to treatment.[50]

Although Parkinson's symptoms are observed in the areas at high risk for ALS/PDC and among individuals exposed to a by-product of meperidine-analog synthesis,[51,52] the vast majority of classical Parkinson's disease cases are believed to have a multifactorial etiology. There is sufficient evidence that the more common form of Parkinson's disease occurring after the age of 50 is not a directly inherited disease,[50] but this does not rule out the possibility of unidentified genetic polymorphisms as contributing factors in Parkinson's disease etiology. Among the environmental risk factors that have been associated with Parkinson's disease are rural living, well-water consumption, and pesticide use; yet the identification of specific agents has not been consistently reported.[53]

Magnetic field exposure has not been among the several environmental risk factors suggested to have an association with Parkinson's disease. The electric utility cohort, cited above as demonstrating a slight association between magnetic field exposure

and ALS and Alzheimer's disease deaths, failed to find such an association when considering Parkinson's disease.[14] Again, this study analyzed both duration of work in exposed jobs and estimated magnetic field exposures. A death certificate-based case-control study reported risk estimates near unity when looking at the broad grouping of electrical workers and within more specific magnetic field-related job titles, yet there were modest, imprecise risks for power plant operators and telephone installers and repairers (OR and 95% CI = 2.1 (0.9 – 4.7) and 1.5 (0.7 – 3.0), respectively.[13] Although there are not many studies specifically exploring a potential relationship between magnetic fields and Parkinson's disease, there is no epidemiologic or etiologic evidence to support such a relationship. Since one would expect a similar proportion of magnetic field-exposed individuals among Parkinson's disease cases and controls, Parkinson's disease would be an appropriate neurological disease to use as a negative comparison analysis in this study.

2.1.4 Brain cancer

One of the original studies on occupational magnetic field exposure demonstrated a significantly elevated proportionate mortality ratio for brain cancer among workers employed in nine occupations considered to have magnetic field exposure.[54] Although brain cancer had a statistically significant elevated PMR, this was not an *a priori* finding, and brain cancer was only one of 158 causes of death considered in this study. Numerous subsequent studies have investigated occupational magnetic field exposure as a risk factor for brain cancer. Among the case-control studies that have been conducted, five were unable to demonstrate increased risk of brain cancer due to magnetic field-related

occupations.[55-59] Seven case-control studies, four of which achieved statistical significance at the 95% confidence level, had relative risk estimates of 1.4 to 3.9.[60-66]

These studies and several others, both cohort and case-control designs, were combined in a meta-analysis.[8] The pooled data suggest a 10 to 20% increase in relative risk, and a combined analysis of six studies using low, medium, and high exposure categories suggested a dose-response relationship.[8] Additional investigations of occupational exposures conducted since this meta-analysis offer mixed results. A Swedish study among individuals living within 300 meters of a transmission line found no increased risk of brain tumors for residential or occupational exposures separately.[67] A non-significant increased risk for certain astrocytomas was observed for individuals with both residential and occupational magnetic field exposure above 2.0 mG, yet this finding was based on only 3 cases.[67] Two case-control studies nested with cohorts of electric utility workers in England and Denmark found no association between death from brain cancer and magnetic field exposure, based on 112 and 72 cases, respectively.[68,69] Another investigation in England found nearly a 20% increase in risk of malignant brain cancer for men under 65 whose primary occupation was within one of 12 job groups identified by the researchers as electrical work.[70]

Case-control studies based on United States death certificate data have more consistently demonstrated a relationship between brain cancer and occupations with high magnetic field exposures.[60-62,64] The ORs for the four positive studies range from 1.4 to 3.9, all of which were statistically significant at the 95% confidence level. A death certificate-based investigation of brain cancer deaths in Maryland reported increases in the odds ratios over four exposure levels with suggestion of a linear trend.[60] Another

study in Texas reporting a postive association between magnetic field-related occupations and brain cancer demonstrated a highly significant linear trend using the same method of four exposure levels.[61] A study of communities in three different states, also using death certificates for case ascertainment and control selection, interviewed next of kin for more specific job exposure information.[62] Although the authors were primarily exploring exposures in the higher microwave to radiofrequency range, they were able to identify a statistically significant trend for total duration of exposure among electrical workers.[62] Finally, a death certificate study covering 16 states reported a slight but significant increase in risk using a list of 14 electrically-related occupations as a means of assessing exposure.[64] The only death certificate study conducted in the United States reporting a lack of association between magnetic field-related occupations and brain cancer was based on an industry grouping of communication and utilities rather than specific job titles.[58] These five studies, together with a death certificate study conducted in England,[56] provided a statistically significant pooled risk estimate of 1.5.[8] Thus, the case control studies based on death certificate data in several different states consistently demonstrated a slight to moderate increased risk for brain cancer among individuals with magnetic field-related job titles.

2.2 Abeta as a biological marker for epidemiological studies of Alzheimer's disease risk

There are some limitations in using the case-control design to investigate potential environmental exposures as risk factors for Alzheimer's disease. Given that Alzheimer's disease is a disease of long duration and imprecise diagnosis, it is difficult to establish causality when identifying antecedent occupational or environmental exposures. The use of a biological marker that infers future risk for development of Alzheimer's disease in combination with real-time exposure assessment could contribute to epidemiological investigations of this disease. In this section, we describe the role of Abeta in Alzheimer's etiology, the significance of this protein in circulating soluble form, and the potential for Abeta as a marker for the future presentation of Alzheimer's disease.

2.2.1 Description of Abeta

Abeta has been implicated in playing a role in Alzheimer's disease because it is one of the principal components found in the senile plaques of the brains of Alzheimer's patients.[71-75] Abeta occurs in various lengths, the most important of which are Abeta(1-42) and Abeta(1-40), the primary isoforms found as fibrillar deposits in senile plaques and cerebrovascular lesions of Alzheimer's patients. Abeta is a proteolytic cleavage product of the transmembrane amyloid precursor protein (APP) found in neuronal cells as well as in circulating lymphocytes and platelets.[76,77] APP is believed to play a role in neuronal cell survival, repair of cell membranes, and stimulation of dendritic and synaptic outgrowth.[78,79] Soluble APP can be secreted when the protein is cleaved by an unidentified enzyme, termed α-secretase (see Figure 2.1).[80] This enzyme cleaves the APP at amino acid 16 of Abeta,

thereby precluding amyloid formation. Alternatively, soluble Abeta has been shown to be a product of APP during normal metabolism, following cleavage at the amino and carboxyl terminal ends of the protein by β-secretase and γ-secretase, respectively (See Figure 2.1).[81,82] The predominant forms of this protein are Abeta(1-40) and Abeta(1-42), with the latter being more likely to undergo a conformational change to an insoluble β-pleated structure and seed the deposition of amyloid deposits.(77) The release of Abeta is dependent upon cleavage within the lipid bilayer section of APP, possibly via an endocytotic process involving acidic compartmentalization into lysosomal pathways.[83] Thus, factors affecting the compartmentalization and processing of surface proteins can influence the proteolytic pathway for APP.

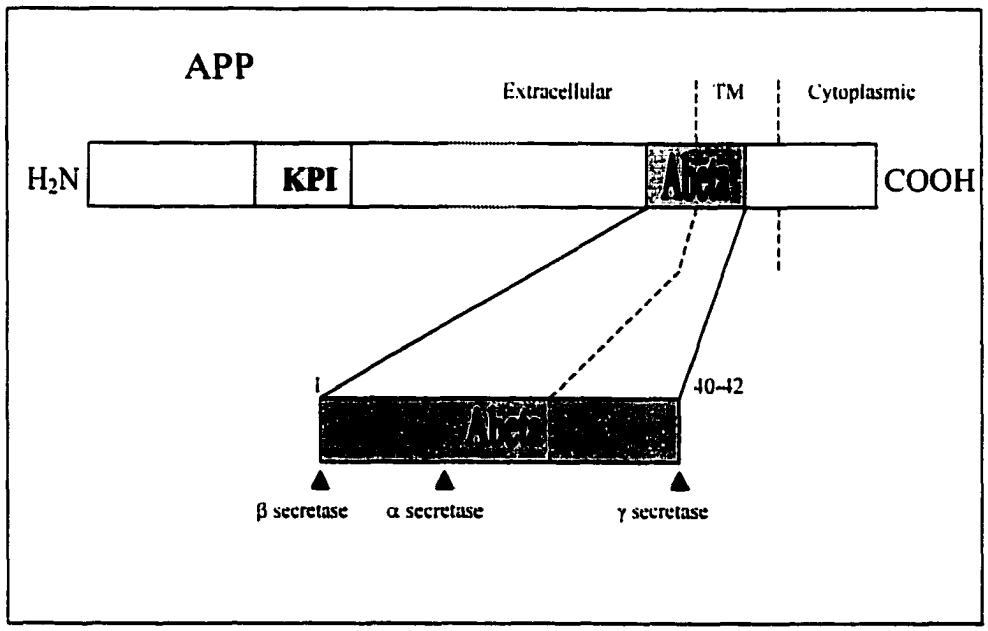

Figure 2.1. Structural organization of the amyloid precursor protein (APP). Production of the Abeta protein requires cleavage within the transmembrane (TM) domain of the APP. KPI = Kunitz protease inhibitor. Adapted from Checler F. Processing of the b-amyloid precursor protein and its regulation in Alzheimer's disease. J Neurochem 1995;65:1431-1444.

The importance of Abeta in the etiology of Alzheimer's disease has been demonstrated in studies of genetic risk factors. Family history of dementia and Down's syndrome have been consistently identified as risk factors for Alzheimer's disease.[29] Persons with Down's syndrome usually develop the pathological lesions of Alzheimer's disease in the third or fourth decade of life. This is believed to be a consequence of being trisomic for chromosome 21, resulting in overexpression of the gene that codes for the APP.[84] An examination of the brains of Down's syndrome patients demonstrates that the presence of high levels of Abeta in its soluble form is antecedent to plaque formation. Compared with undetectable levels in age-matched controls, elevated levels of soluble Abeta were detected in the brains of Down's syndrome subjects both in the absence, subjects less than 20 years, and the presence, subjects over 20 years, of amyloid plaques.[85]

In addition to studies of Down's syndrome patients, genetic studies provide a strong link between Abeta and inherited forms of Alzheimer's disease. A number of point mutations on the chromosome 21 gene that codes for APP have been linked with early onset familial cases of Alzheimer's disease.[21] Elevated secretion of soluble Abeta(1-42) has been observed in neuroblastoma cells transfected with a construct expressing an APP mutation associated with familial Alzheimer's disease.[86] Higher levels of secreted Abeta were also observed in peripheral cells from individuals with familial Alzheimer's disease mutations on the APP gene.[87] Other point mutations associated with familial Alzheimer's disease occur on the chromosome 14 and chromosome 1 genes coding for presenilin proteins. Both *in vivo* analysis of plasma levels among carriers of these presenilin mutations

and *in vitro* analysis of skin fibroblasts cultures expressing these mutations demonstrated dramatic elevations in levels of Abeta(1-42) compared to those of controls.[88-90]

2.2.2 The importance of circulating soluble forms of Abeta

Recent findings support the relevance of studying circulating soluble Abeta in association with Alzheimer's disease pathogenesis. Abeta deposits have been found in non-neural tissue of Alzheimer's patients, including skin, subcutaneous tissue and intestine, suggesting a role for circulating forms of Abeta and APP in Alzheimer's disease pathogenesis.[91,92] Moreover, soluble forms of Abeta(1-40) and Abeta(1-42) were isolated from the cerebrospinal fluid and plasma of Alzheimer's patients.[93-96] The presence of these important isoforms in circulating soluble forms suggests similarities between Alzheimer's disease and other amyloid related diseases. Amyloid A and immunoglobulin light-chain related amyloidosis involve circulating soluble amyloid precursors, and the presence of Abeta in both soluble and fibrillar forms is similar to systemic senile amyloidosis in which the soluble form of the amyloid precursor can undergo conformational change to a beta-pleated fibrillar structure.[97] As in these other forms of amyloid disease, the aggregation of Abeta in Alzheimer's disease is concentration dependent.[98]

2.2.3 Using plasma Abeta levels as an indicator for future risk of Alzheimer's disease

Concentrations of soluble Abeta found in blood come from both circulating and neuronal sources. Platelets were found to be a major source of plasma APP among both

Alzheimer's disease patients and controls.[76] Stimulated human peripheral mononuclear blood leukocytes were also shown to be a source of the soluble form of circulating APP.[77] Neuronally-derived Abeta can also contribute to blood levels. When high levels of soluble Abeta are infused into the cerebrospinal fluid (CSF) of adult rats, there is a rapid clearance of the protein from the CSF-brain system across the capillaries and into blood.[99] Alternatively, it has been demonstrated that neuronally-derived Abeta can be removed via the periarterial spaces to the cervical lymph nodes and finally into the venous circulation.[100]

Three independent lines of evidence suggest the relevance of using Abeta levels in blood as an indicator for the future risk of Alzheimer's disease. First, plasma Abeta is elevated among individuals genetically predisposed to Alzheimer's disease. People with Down's syndrome which, as mentioned previously, involves the overexpression of APP were shown to have levels of plasma Abeta two- to three-fold higher than that of controls.[101,102] Two studies described individuals with the familial Alzheimer's disease APP mutations as having two-fold higher levels of plasma Abeta(1-42) compared to control subjects.[89,103] In each of these studies, elevated levels of Abeta(1-42) were observed among presymptomatic carriers of the APP mutations, as well as among those expressing the clinical symptoms of Alzheimer's disease.[89,103]

While higher levels of plasma Abeta are clearly associated with familial Alzheimer's disease, there is less of a direct correlation with sporadic forms of Alzheimer's disease. Studies comparing sporadic Alzheimer's disease patients with non-demented controls found no overall difference in plasma Abeta levels.[89,94,104] In each of these studies, however, a subset of sporadic Alzheimer's disease cases, approximately

10%, had plasma Abeta levels similar to that found in individuals with familial Alzheimer's disease mutations.[89,94,104] Although the majority of sporadic Alzheimer's disease cases do not have elevated plasma Abeta levels, these levels could be affected by the pathological lesions associated with Alzheimer's disease. Indeed, CSF levels of soluble Abeta(1-42) in Alzheimer's disease patients were reduced in comparison to non-demented controls [95]. Soluble Abeta in clinically symptomatic Alzheimer's disease patients may be less likely to be present in the blood due to a disruption in the normal clearance mechanism and/or an affinity for deposition on aggregated Abeta in the senile plaques and the cerebrovasculature. While this may account for low Abeta plasma levels in the majority of individuals with sporadic Alzheimer's disease, the elevated plasma levels observed in familial Alzheimer's disease patients and in some sporadic Alzheimer's disease patients suggests the continued influence of genetic and/or environmental factors on peripherally-derived Abeta.

The second line of evidence implicating circulating soluble Abeta in Alzheimer's disease pathology is its ability to be transported across the blood brain barrier (BBB). The BBB controls the exchange between the CSF and brain interstitial fluids and the circulating substances carried by the blood. The BBB consists of a monolayer of cells bound by tight junctions, thereby eliminating intercellular gaps. Circulating Abeta could affect the CNS by crossing the BBB and gaining direct access to neuronal cells, or it could disrupt the BBB and affect the exchange of substances. Zlokovic et al.[105], using guinea pig brains, demonstrated high rates of Abeta transport across the blood brain barrier when compared with sucrose. The passage of radioiodinated Abeta(1-40) across

the BBB was also shown using both a mouse perfusion model and intravenous injection.[106-108]

This property of blood-borne Abeta may be consistent with the apoE4 polymorphism risk factor for Alzheimer's disease. Studies utilizing perfused guinea pig brain implicated the role of apolipoproteins in controlling brain uptake of circulating Abeta.[109,110] Following carotid arterial infusion, brain uptake of Abeta complexed with apolipoprotein-J (apoJ) was 4-fold higher than uptake of Abeta alone, whereas uptake of Abeta complexed with apolipoprotein-E (apoE) was 30-fold lower than for Abeta alone.[109] In a study of the isoform-specific effects of apoE, only Abeta bound to apoE4 demonstrated significant transport across the BBB, yet transport for Abeta bound to apoE2 and apoE3 was negligible.[110] These findings, together with indications that isoforms apoE2 and apoE3 bind Abeta more readily than apoE4,[111,112] offer a possible connection between circulating Abeta and the role of apoE4 as a genetic risk factor for Alzheimer's disease.

The third line of evidence indicating a relationship between circulating Abeta and Alzheimer's disease involves the cerebrovascular pathology associated with Alzheimer's disease. Although the BBB studies cited above demonstrate passage of Abeta into the parenchymal space, the majority of Abeta in these experiments was sequestered by the brain capillaries.[106,107] Thus, in addition to the possibility that circulating Abeta may contribute to the formation and/or growth of senile plaques, peripherally-derived Abeta may play a stronger role in the cerebrovascular pathology associated with Alzheimer's disease. Cerebral amyloid angiopathy (CAA) is reported to be present in 83% to greater than 90% of Alzheimer's disease cases and consistently present in Down's syndrome.[113-

[115] CAA consists of extracellular accumulation of Abeta deposits in the cerebrovasculature. Deleterious changes in the microvascular wall associated with CAA include thickening of the basement membrane, degeneration of endothelial cells, and reduction of the vascular lumen.[116] These effects may impair the normal delivery of nutrients to the neuronal or glial cells or disrupt the outflow of CNS waste. It is not clear, however, whether vascular changes in Alzheimer's disease precede or follow neuronal loss and degeneration.

In addition to the prevalence of CAA and alterations in the vascular wall in conjunction with Alzheimer's disease, soluble forms of Abeta are vasoactive. While Abeta produces endothelial cell toxicity at high concentrations, the observed vasoactive effects of Abeta occur at physiological levels.[117] The vasoactivity of Abeta is mediated by the presence of other vasoactive forces. Increased contraction to the vasoconstrictor endothelin-1 (ET-1) and diminished relaxation to the vasodilator bradykinin were observed in both rat aorta and bovine cerebral arteries treated with soluble Abeta(1-40).[118,119] *In vivo*, rats intra-arterially infused with soluble Abeta(1-40) demonstrated decreased cerebral blood flow and increased vascular resistance.[120] This vasoactive property of Abeta was preferentially confined to the cerebral microvasculature, as the same study demonstrated no effect of Abeta infusion on blood flow in the heart or kidney.[120] Moreover, the genetic Alzheimer's disease risk factor, apoE, was shown to influence Abeta vasoactivity in an isoform-specific manner. Compared to Abeta alone, the rate of constriction induced by ET-1 was enhanced when Abeta was combined with apoE4 but not significantly enhanced when combined with apoE3 or apoE2.[121] The enhanced constriction and delayed relaxation by Abeta observed in these studies suggests

that peripherally-derived Abeta could play a role in cerebral hypoperfusion and, consequently, in Alzheimer's disease pathogenesis.

2.2.4 Summary of Abeta as biological marker

Whether the soluble Abeta found in blood comes primarily from neuronal cells of the CSF-brain system or from circulating carriers of APP. environmental factors that contribute to amyloidogenic processing of APP can be assessed by their influence on Abeta concentrations in this matrix. Three lines of evidence indicate the relevance of peripherally-available Abeta in Alzheimer's disease. First, individuals that are genetically predisposed to Alzheimer's disease and some patients with sporadic Alzheimer's disease exhibit elevated levels of circulating Abeta. Second, demonstrations of the passage of Abeta across the BBB suggest that circulating forms of this protein contribute to amyloid lesions, perhaps in conjunction with the apolipoprotein E polymorphism associated with Alzheimer's disease. Finally, peripherally-derived Abeta contributes to the cerebrovascular lesions of Alzheimer's disease, and the vasoactivity of Abeta could play an indirect role Alzheimer's disease pathology. The study of factors relevant to the increased concentration of circulating Abeta is, therefore, important in understanding the etiology of the disease.

2.3 Biological basis for relationship between Abeta and magnetic field exposure

In vivo and in vitro laboratory studies have been a major focus of magnetic field research since the initial epidemiologic relationship was described in 1979. This research has lead to a partial understanding of the complexity of biological responses to these fields. Some of the observed biological effects of magnetic field exposure may be relevant to APP processing and Abeta release and are discussed in the following section.

2.3.1 Magnetic field effects on calcium homeostasis and the association with Abeta

One potential mechanism whereby magnetic fields could influence APP processing is through a disruption of calcium homeostasis. Concentrations of intracellular calcium (Ca^{2+}) are usually maintained at levels four orders of magnitude lower than in the extracellular space. This gradient is maintained by calcium pumps and ion exchangers that transport ions across the plasma membrane and the intracellular membranes of the mitochondria and the endoplasmic reticulum.[122] Intracellular Ca^{2+} plays an important role as a second messenger in cell systems and in regulating membrane permeability, transport, and secretion.[123-125] Thus, factors influencing calcium movement across cell membranes could have an effect on the processing of membrane resident proteins such as APP.

The role of magnetic fields in perturbing calcium homeostasis has been demonstrated in numerous experimental settings. Bawin and Adey first demonstrated an efflux of Ca^{2+} from isolated chick brain tissue when exposed to radio frequency and extremely low frequency magnetic fields.[126] These experiments were repeated by Blackman who identified the requirement for specific windows of field intensity and frequency.[127] Other studies have found a Ca^{2+} influx among rat thymocytes and human

peripheral blood mononuclear cells exposed to magnetic fields.[128-131] Intriguing yet imperfect theoretical models based on ion cyclotron resonance have been advanced to explain these observations.[132] Although these *in vitro* studies have shown calcium oscillation effects to be dependent upon specific ranges of frequency, field flux density, and orientation, it is important to note that these fields are eliciting biological responses in the extremely low frequency range present in many occupational settings.

The effects of increased intracellular calcium include altered protein phosphorylation, proteolysis, and membrane lipolysis.[133] All these actions can be important in the processing of a transmembrane protein such as APP. Recent studies have shown an association between intracellular calcium levels and the production of Abeta.[134,135] Buxbaum et al.[134] described the means by which calcium ion concentration participates in the phospholipase C pathway of APP processing. Their findings indicated that increased intracellular Ca^{2+} levels inhibit Abeta production, yet certain concentrations of thapsigargin which increase cytoplasmic levels of Ca^{2+} resulted in increased production of Abeta.[134] Querforth and Selkoe[135] have also shown a positive relationship between intracellular calcium and Abeta. Using a calcium ionophore on human kidney cells, the authors demonstrated that Ca^{2+} influx promotes Abeta production. Higher Abeta production was also associated with the introduction of caffeine which heightens cytoplasmic levels of calcium by releasing it from intracellular stores.[135] These observations suggested that a calcium-requiring protease is involved in the metabolic pathway generating Abeta.[135] While these studies are not conclusive, they do implicate calcium ions as a potential factor in releasing the primary component of amyloid plaques from APP.

2.3.2 Magnetic field-induced transcriptional effects and the association with Abeta

Cells exposed to magnetic fields have been shown to undergo alterations in protein biosynthesis and stimulation of transcription factors. Magnetic fields, applied to four different cell lines, have been shown to increase the steady state levels of transcripts coding for *c-fos* and *c-myc*,[136-139] although other studies have been unable to replicate these findings.[140,141] The potential link between magnetic field regulation of signaling pathways and APP processing is found in the relevant response regions of *c-fos*. Deletion analysis of the *c-fos* gene indicates that the region responsive to magnetic field stimulation includes the site for activation protein-1 (AP-1).[138] Following magnetic field exposure, human HeLa cells, transfected with a *c-fos* promoter-chloramphenicol transferase (CAT) construct, showed heightened CAT protein levels when the construct included the AP-1 region and essentially no change over controls when the AP-1 region was deleted.[138] Similarly, four human cell lines exposed to magnetic fields expressed AP-1 binding activity in electrophoretic mobility shift assays.[142] The APP gene contains a regulatory region homologous to the AP-1 recognition sequence, and AP-1 binding has been shown to promote APP gene expression.[143] The responsiveness of APP gene expression to phorbol 12-myristate 13-acetate (PMA) was dependent upon the AP-1 binding site, and mutation at this site rendered APP unresponsive to PMA stimulation.[143] Thus, the magnetic field activation of AP-1 binding through transcription factors may trigger the overexpression of APP.

Magnetic fields have also been shown to induce the transcription of heat shock proteins (hsp),[144-147] suggesting an association between magnetic fields and cellular heat shock response. Changes in protein distribution in dipteran salivary gland cells exposed to a

60 Hertz (Hz) magnetic field at 80 mG matched the response of these cells to sustained heat shock at 37°C.[145] Similarly, levels of hsp70 transcripts in human HL-60 cells were elevated following exposure to 60 Hz at various field strengths and exposure times.[146] When yeast cells are exposed to 60 Hz magnetic fields, increases in the transcript levels for SSA1, a heat shock gene, were observed.[147] It must be noted, however, that each of these studies indicating a heat shock response were conducted in the same laboratory; and a separate laboratory using different exposure methods was unable to replicate these findings.[140]

There are several associations between heat shock cellular response and APP processing. Heat shock proteins function in the early events of protein processing by facilitating the transport of polypeptides and promoting proteolysis of altered proteins.[148,149] The APP gene contains a heat shock consensus element, and heat shock proteins also have been known to accumulate in the senile plaques of Alzheimer's disease patients.[148] Recently, it was shown that APP-transfected cells exposed to heat shock undergo abnormal processing and compartmentalization.[150] In this experiment over 90% of the glioblastoma and neuroblastoma cells exposed to 44°C for 30 minutes demonstrated increased expression of APP and accumulation in a Golgi-like pattern.[150] This pathway was different from normal secretion processing of the soluble form of APP and suggested possible mechanisms for amyloidogenic production through miscompartmentalization.[150] Thus, magnetic fields may affect Abeta processing by triggering AP-1 binding and the subsequent overexpression of APP and/or by stimulating a heat shock response and the potential misprocessing of APP.

2.3.3 Magnetic fields and melatonin

The indoleamine hormone melatonin, N-acetyl-5-methoxytryptamine, is synthesized in the pineal gland from serotonin after acetylation and methylation. In addition to its role in the biologic regulation of circadian rhythms, it is thought to be involved in oncostatic effects, immune stimulation, reproduction, and senescence.[151] Suppression of melatonin due to magnetic field exposure has been proposed as a potential biological mechanism explaining the observed association between magnetic fields and increased risk of cancer.[152] Studies of this relationship in humans exposed to magnetic fields in a controlled laboratory environment offered inconsistent results. Using a laboratory exposure chamber, individuals with low baseline melatonin demonstrated a suppression in response to magnetic field exposure between 23:00 and 7:00 compared with a group of sham-exposed controls.[153] This effect was not observed in subsequent studies using similar exposure methods with individuals serving as their own controls.[153-155] Another laboratory study in humans, timing exposure to coincide with the predicted onset of the evening rise in melatonin concentration, found a delay in onset and, presumably, lower total melatonin production for 40% of the subjects.[156]

Recent studies of occupationally exposed humans suggested an association between magnetic fields and melatonin. Among 142 male electric utility workers, individuals in the highest quartile of temporally stable magnetic field exposure had decreased post-work levels of the urinary melatonin metabolite 6-OHMS on the second and third days of exposure monitoring compared with individuals in the lowest quartile.[157] This effect was more pronounced when restricting to individuals in the lowest quartile of workplace light exposure.[157] The same population demonstrated

decreases in overnight 6-OHMS excretion among individuals exposed to temporally stable magnetic fields both at work and at home.[158] A population of electric railway engineers with high magnetic field exposures had reductions in post-work afternoon 6-OHMS concentrations compared with samples taken prior to the start of their work week.[159]

2.3.4 Melatonin and its association with Alzheimer's disease and Abeta

One of the functions of the hormone melatonin is regulation of the circadian rhythm with peak levels at night returning to baseline levels during the day. The production of melatonin declines progressively with age, and peak night-time levels in older individuals are considerably lower compared to younger individuals.[160] In addition to this natural decline in melatonin levels with aging, there appears to be a further disruption of circadian rhythmicity among patients suffering from dementia. One post-mortem study evaluating human pineal glands found no variation in melatonin levels among Alzheimer's disease patients by time of death.[161] This was in sharp contrast to the high variation in melatonin levels by time of death among controls, yet the control group had a much lower age distribution than the Alzheimer's disease group.[161] Studies analyzing serial blood samples taken over 24 hours showed that the melatonin circadian profile of Alzheimer's disease patients compared to age-matched controls was flattened due to a reduction in peak night-time levels.[162,163] Measurements of melatonin in cerebrospinal fluid was also found to be 33% lower among Alzheimer's disease patients compared to controls with some suggestion of a correlation between melatonin levels and a measure of severity of demetia.[164]

It is difficult to determine if the above-cited studies indicating reduced melatonin levels among Alzheimer's disease patients were a cause or a consequence of dementia, yet recent studies indicate that melatonin may influence Abeta production, both directly and indirectly. Levels of the soluble form of APP (sAPP) were analyzed in neuroblastoma and pheochromocytoma (PC12) cells, and a sharp decrease in the secretion of sAPP in conditioned medium was observed among cells treated with melatonin.[165] A 54% reduction in APP mRNA was also observed in melatonin-treated PC12, but not neuroblastoma, cells.[165] In a related study, the secretion of Abeta(1-40) and Abeta(1-42) into the conditioned medium was reduced 25-80% following treatment of neuroblastoma cells with different concentrations of melatonin.[166]

The relationship between melatonin and Abeta processing is further demonstrated by mechanistic studies. One of the reported mechanisms whereby aggregate Abeta can induce neuronal death is through its ability to promote calcium ion influx and/or impair calcium extrusion.[167-169] As illustrated previously, non-homeostatic levels of intracellular calcium also could promote the abnormal processing of APP.[134,135] Melatonin has been shown to have a cytoprotective effect on neuroblastoma cells by limiting this Abeta-induced elevation in intracellular calcium.[170] This function of melatonin is consistent with the observation that melatonin stimulates the activity of the calcium pump in a preparation of rat cardiac sarcolemma.[171] Thus, lowered concentrations of melatonin may hinder the effectiveness of this hormone in limiting the ability of Abeta aggregates to self promote the release of Abeta via Ca^{2+} influx.

The second mechanism whereby melatonin could affect Abeta processing is related to its role as a protector against oxidative stress. *In vitro*, melatonin was shown to

be a strong scavenger of hydroxyl radicals generated by hydrogen peroxide (H_2O_2), and *in vivo*, melatonin was shown to inhibit saffrole-induced DNA adduct formation.[172,173] Melatonin has also been shown to stimulate antioxidant enzymes, specifically glutathione peroxidase and superoxide dismutase, in neural tissue from rats and chicks.[174-176] Although these findings were based on the administration of pharmacological doses of melatonin, a study of pinealectomized rats indicated that deficiency in endogenous levels of melatonin results in greater neuronal degeneration following both focal brain ischemia and kainic acid-induced seizure.[177]

Oxidative stress has been implicated in promoting the production and release of APP and Abeta.[178-180] Mammalian lenses which normally produce low levels of Abeta associated with aging and retinal degeneration demonstrated elevated expression of APP and Abeta following treatment with either H_2O_2 or ultraviolet radiation.[178] In another study, an Abeta-containing fragment was found to be highly associated with H_2O_2-induced apoptosis of neuroblastoma cells.[180] The authors suggest a vicious cycle mechanism whereby oxidative stress generates Abeta which, in turn, promotes oxidative stress, leading to an apoptotic build-up of Abeta.[180] Finally, the glycation of paired helical filament tau, the principle component of the neurofibrillary tangles associated with Alzheimer's disease, results in the generation of oxygen free radicals and the subsequent release of Abeta.[179]

In addition to its direct effect on Abeta release, oxidative stress plays a complementary role with the magnetic field-induced biological effects described above. First, oxidative stress can result in disruptions in calcium homeostasis,[133] potentially leading to elevated production of Abeta.[134,135] Second, in addition to directly causing

the abnormal compartmentalization of lysosomal enzymes which affect APP processing, oxidative stress can lead to abnormal protein processing through induction of heat shock proteins.[181] As mentioned previously, the heat shock response has been shown to be involved in increased secretion and altered processing of APP.[148,150] Finally, the binding properties of AP-1 are elevated under conditions of oxidative stress,[178] and activation of AP-1 has been shown to promote APP gene expression.[143] Thus, given the potential for oxidative stress to moderate the processing of Abeta, melatonin, in as much as it serves as a protector against oxidative stress, may be an important factor in this relationship.

2.3.5 Summary of magnetic field effects and their relationship to Abeta

Each of the above described magnetic field-induced biological effects, although imperfectly understood and inconsistently observed, may independently and in combination with each other affect Abeta processing and expression. These relationships are presented in Figure 2.2. First, protein processing and secretion is sensitive to alterations in intracellular Ca^{2+} concentrations. Disruptions in calcium homeostasis have been shown to promote the release of Abeta, and both magnetic fields and oxidative stress can alter calcium flux. Second, cellular stress, due to thermal or oxidative conditions, results in abnormal processing and miscompartmentalization of APP. The APP gene contains a heat shock consensus element, and heat shock proteins also have been known to accumulate in the senile plaques of Alzheimer's disease patients. Cells exposed to magnetic fields simulate a heat shock response, and cells exposed to the superoxide free radical respond by expressing heat shock proteins. Third, AP-1 binding promotes APP gene expression,

allowing increased opportunity for Abeta production. Both magnetic fields and oxidative stress can stimulate AP-1 binding. Finally, melatonin has been shown to influence Abeta production *in vitro*, and the known properties of melatonin as an antioxidant and a moderator of calcium homeostasis suggest an indirect role in APP processing. Exposure to certain magnetic fields appears to cause a reduction in melatonin biosynthesis, thereby limiting the action of this hormone in mediating Abeta release.

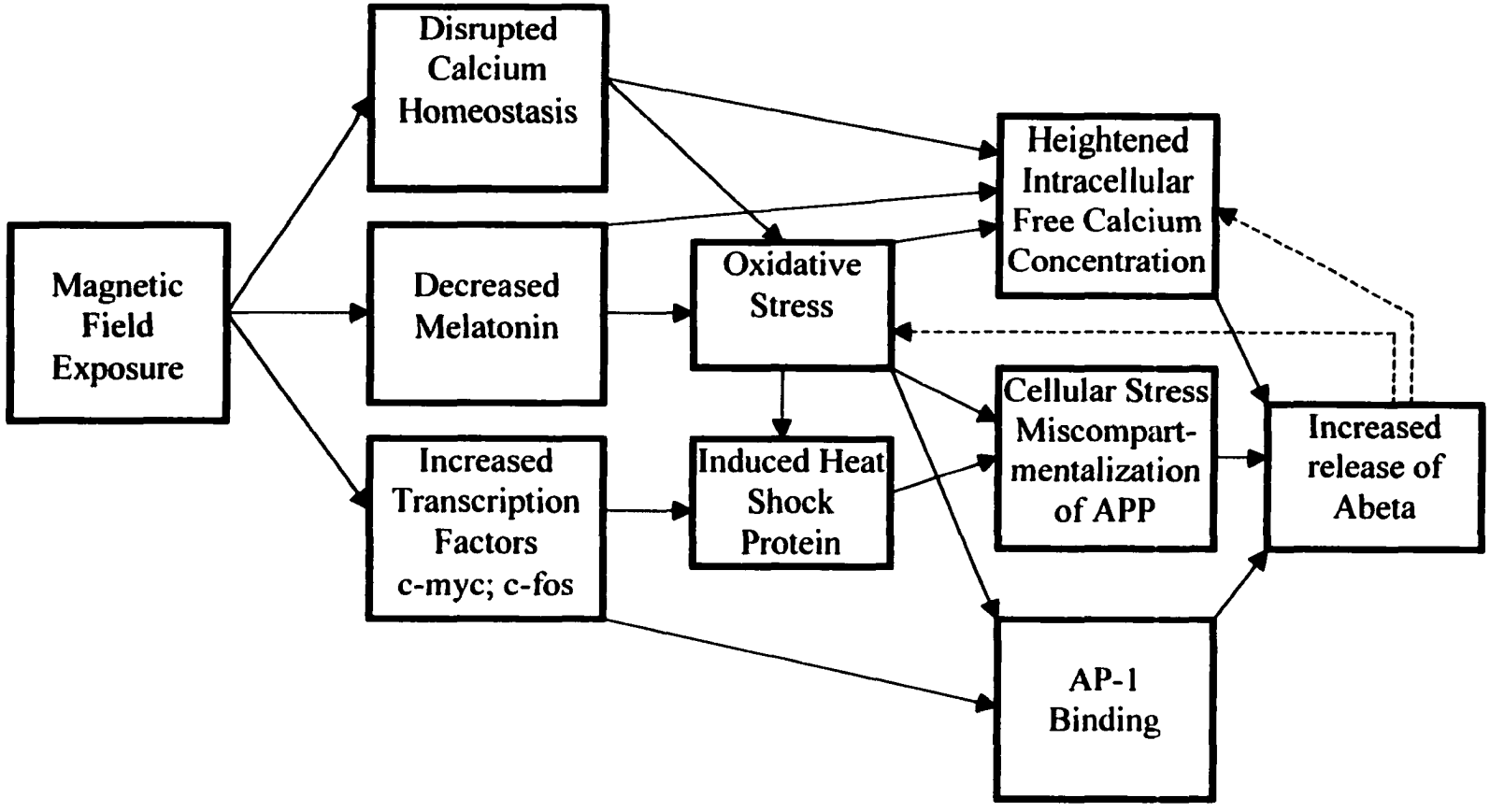

Figure 2.2. Magnetic field-induced biological effects and potential mechanisms for inducing elevated amyloid beta protein (Abeta). Magnetic field exposure may result in decreased melatonin, irregular calcium flux, and induction of transcription factors. These properties can influence calcium homeostasis, protein processing, and AP-1 binding; thereby affecting Abeta release. Aggregated Abeta may also self-promote the release of additional soluble Abeta through its promotion of oxidative conditions and influence on calcium homeostasis.

CHAPTER 3

ASSESSMENT OF OCCUPATIONAL EXPOSURE TO MAGNETIC FIELDS IN CASE-CONTROL STUDIES OF NEUROLOGICAL DISEASES

3.1 Introduction

Several recent epidemiological studies of occupational exposure to magnetic fields and neurodegenerative disease have found evidence of increased risk associated with these exposures. Individuals employed in occupations considered to have medium to high exposure to magnetic fields have been shown to be at increased risk for Alzheimer's disease and amyotrophic lateral sclerosis.[9-18] In an effort to confirm and expand the findings of previous studies, we conducted death certificate-based analyses of these outcomes. As part of the design, we compared the use of three different approaches to exposure assessment: a dichotomous grouping of electrical versus non-electrical occupations; a three tiered grouping of potential magnetic field exposure based on a combination of job title and industry; and quartiles of exposure based on estimated mean magnetic field values from a job exposure matrix (JEM).

Numerous studies have investigated occupational magnetic field exposure as a risk factor for brain cancer, and a meta-analysis found a 10-20% increase in relative risk.[8] Although more recent investigations provided mixed results,[67-70] previous death certificate-based studies using job title for exposure assessment have consistently shown

a positive association.[60-62,64] To test the validity of our exposure assessment, we hypothesized that an association between brain cancer and occupational exposure to magnetic fields should exist in this data set.

By contrast, occupational magnetic field exposure has not been among the several environmental risk factors associated with Parkinson's disease, although the number of studies exploring this relationship is limited. Both a retrospective cohort study and a death certificate-based case-control study failed to find an association between Parkinson's disease and occupational magnetic field exposure.[13,14] Since one would expect a similar proportion of cases of Parkinson's disease and controls to have occupational magnetic field exposure, an analysis of Parkinson's disease was included in this study as a comparison outcome.

3.2 Methods

Death certificate data were collected from the Vital Statistics Unit of the Colorado Department of Public Health and Environment. Four separate case-control sets were formed from among recorded male deaths for the years 1987 to 1996. Cases were selected based on any mention of Alzheimer's disease (ICD Code 331.0), amyotrophic lateral sclerosis (ICD Code 335.2), Parkinson's disease (332.0), or brain cancer (ICD Codes 191.0-191.9 (malignant brain neoplasm), 225.0 (benign brain neoplasm), and 239.6 (brain neoplasm of unspecified nature)). In an attempt to reduce the number of early onset cases of presumed familial origin, persons with Alzheimer's disease were restricted to an age at death of 60 years or more. Cases of amyotrophic lateral sclerosis,

Parkinson's disease, and brain cancer were selected with an age at death restriction of 30 years or more to allow for sufficient occupational exposure.

Male controls were selected separately for each disease group and were frequency matched by five-year age intervals and year of death. Due to the small number of cases of amyotrophic lateral sclerosis, we used a 4:1 control to case ratio for this analysis. A 1:1 control to case ratio was employed for all other case groups. These ratios provided 80% power to detect on odds ratio of 2.0 at the 95% confidence level, for a dichotomous exposure. Any death certificates with mention of leukemia or breast cancer were ineligible for control selection since magnetic fields are a putative risk factor for these diseases. Death certificates with any mention of the four diseases of interest were also ineligible for control selection.

All death certificates included individuals' primary lifetime occupation and industry, classified with three-digit occupation and industry codes based on the 1980 U.S. Bureau of Census Classified Index of Industries and Occupations.[182] In order to compare the sensitivity of associations with different methods for magnetic field exposure assessment, we assessed risk using three exposure classification systems. The first method applied a dichotomous exposure classification for electrical occupations used previously to identify individuals with occupations having probable high exposure to magnetic fields in several case-control studies of brain cancer.[13,54,64] The electrical occupations used in these studies include electrical and electronic technicians and engineers, repairers of electronic equipment, telephone and telephone line installers and repairers, electricians, electric power installers and repairers, supervisors of electricians and power transmission installers, power plant operators, motion picture projectionists,

and broadcast equipment operators.[13,54,64] All individuals with other occupations were considered to have no magnetic field exposure. The second method used multiple exposure levels and has also been employed in death certificate-based case-control studies of magnetic field exposure and brain cancer.[60,61] This approach classified individuals into one of four exposure categories based on a combination of occupation and industry codes. For this study, the two highest categories (Definite and Probable magnetic field exposure) were combined due to the low number of individuals found in the second tier of exposure (see Appendix A). The third method of exposure assessment was based on a JEM developed at the University of Washington for a study of adult brain cancers. This JEM was later translated and expanded into a Bureau of Census code version for a National Institute of Occupational Safety and Health study of neurodegenerative diseases. Job titles were assigned time-weighted geometric mean magnetic field values based on measured magnetic fields in a large series of observations collected from various sources.[183] Individuals were then divided into quartiles based on their estimated magnetic field exposures. The JEM contained geometric mean values for 85% of the job titles in our data sets, accounting for 79-84% of the individuals.

We performed logistic regression in SAS 6.12 (Cary, NC) on each case-control group for each of three exposure methods. All odds ratios were adjusted for race and a social class variable. Social class was assigned to three levels, based on broad occupational categories. This approach was taken to control for socio-economic status as a potential confounder rather than years of education since data for education was not available for the first two years of our study. There were no substantial changes in risk estimates when education was included in the analyses (see Appendix B).

3.3 Results

Descriptive data for each case-control group are presented in Table 3.1. As expected, the majority of Alzheimer's disease and Parkinson's disease cases were found among the older age groups, whereas the amyotrophic lateral sclerosis and brain cancer cases had a younger age distribution. Amyotrophic lateral sclerosis and brain cancer had different educational and occupational distributions than the other two diseases, which may reflect the age distributions. All four diseases had a slightly higher proportion of deaths recorded in the years 1992 to 1996 which could be due to more sensitive reporting or demographic changes. Analyses of descriptive variables indicated that non-Hispanic whites with high educational attainment and professional and managerial occupations were consistently more likely to have these four diseases listed on their death certificate (Table 3.2). The group of professional and managerial occupations also was less likely to include job titles with high MF exposure. Eleven of the fifteen electrical occupations were included in the referent occupational category of service/labor and others, and the JEM-estimated geometric mean for this referent group was 1.78 milliGauss (mG) versus 1.26 mG for the professional and managerial group ($p < 0.001$).

The average estimated geometric means (in mG) by quartile of job titles using the JEM (method #3) were 2.90, 1.58, 1.09, and 0.67 for the Alzheimer's disease case-control group and were similar for the other disorders analyzed. Estimates of magnetic field intensity exposure using the JEM correlated well with the other methods of exposure assessment. For the Alzheimer's disease case-control group, the estimated geometric mean for electrical versus non-electrical workers (method #1) was 4.03 mG and 1.5 mG,

respectively ($p < 0.001$). The JEM estimated geometric means for the three tiers of exposure based on job title and industry (method #2) were 4.68 mG, 1.97 mG, and 1.38 mG.

In Tables 3.3 and 3.4, crude and adjusted odds ratios for each outcome are shown using three different approaches to exposure assessment. When magnetic field exposure was defined by electrical occupations (method #1), an elevated risk for amyotrophic lateral sclerosis and Parkinson's disease was found (adjusted odds ratios and 95% confidence intervals, 2.30 (1.29-4.09) and 1.55 (0.98, 2.45), respectively). Risk estimates for Alzheimer's disease and brain cancer were not different from unity using this method.

The second analysis used three categories of magnetic field exposure by occupation and industry groupings (method #2). We found estimates similar to those for the dichotomous exposure method for Parkinson's disease with suggestion of a dose-response trend (Table 3.3). Risk estimates for amyotrophic lateral sclerosis also were elevated, although less than for method #1. A weak, imprecise association was found for Alzheimer's disease in the highest category of exposure. There was no indication of increased risk for brain cancer.

The risk estimates for all case groups changed substantially when individuals were assigned to quartiles of exposure based on the geometric means obtained from the JEM (method #3). A slightly elevated risk for brain cancer (adjusted odds ratio and 95% confidence interval, 1.32 (0.92, 1.89)) was found for the highest quartile of magnetic field exposure (Table 3.3). This was the only method of exposure for which an increased risk for brain cancer was suggested. The risk estimates for Parkinson's disease were reduced below those calculated for other methods of exposure and there was no evidence of a

dose response trend by quartile of exposure. No increases in risk were found for Alzheimer's disease or amyotrophic lateral sclerosis when quartile of estimated magnetic field exposure was used to classify cases and controls.

A review of the estimated magnetic field exposures assigned to electrical occupations by the JEM offered a partial explanation for the disparate findings presented above. Table 3.5 lists the 15 electrical job titles traditionally considered to have high magnetic field exposure and their corresponding JEM geometric means. Some of these job titles, such as electric power installers/repairers and power plant operators, were assigned high exposure values, but several within this group of job titles had considerably lower values. Four of these electrical occupations for which there were adequate data did not fall into the upper quartile of estimated exposure based on the JEM. Table 3.6 lists the fifteen job titles with the highest JEM-assigned geometric mean. Ten of these job titles were not included among the electrical occupations.

To further explore the reasons for the discrepancies in the findings with each method of exposure assessment, we conducted additional comparisons of specific job titles within each case-control group. Most of the elevated risk for amyotrophic lateral sclerosis and Parkinson's disease using methods #1 and #2 was due to electrical/electronic technicians and engineers (job codes 213 and 55, respectively) and, to a lesser extent, electricians (job code 575). As illustrated in Table 3.5, job codes 213 and 55 were assigned geometric mean values that were low relative to some of the higher exposure job titles. Thus, it is not surprising that the use of the JEM in the exposure assessment reduced the originally observed elevated risk for amyotrophic lateral sclerosis and Parkinson's disease. A review of the other magnetic field-related occupations used

in method #2 also found welders to be at increased risk of Parkinson's disease. We observed 12 welders with Parkinson's disease versus 6 welders among the controls (adjusted odds ratio and 95% confidence interval, 2.26 (0.85-6.06)).

The use of multiple exposure categories by job title and industry (method #2) was the only exposure assessment method suggesting a modest trend of association for Alzheimer's disease, although the risk estimates were imprecise. A post-hoc review of the occupational distribution of cases and controls indicated that the change in the risk estimate using this method was due primarily to the inclusion of aerospace engineers in the high exposure stratum and the reassignment of electric technicians not employed in the utility or telecommunications industry to a lower stratum.

The odds ratios for brain cancer were close to unity when using categorical groupings based on job title or the combination of job title and industry (methods #1 and #2). Four job titles accounted for a large majority of the individuals classified as exposed under these two methods. There were more than twice as many brain cancer cases as controls with job titles listed as electrical and electronic technicians and engineers (job codes 213 and 55, respectively). However, the inverse was true for electricians and electric power installers and repairers (job codes 575 and 577, respectively). When using the JEM (method #3), the most apparent job title accounting for the slightly elevated risk estimate in the highest quartile of exposure was mechanics (adjusted odds ratio and 95% confidence interval, 2.70 (1.34-5.44)). The JEM-estimated geometric mean for mechanics (job codes 505-508, 515-517, and 534) was 2.3 milliGauss based on 32 observations. This group of job titles was not included among the exposed using method #1 but was included in the second tier of exposure using method #2.

3.4 Discussion

The three methods of exposure assessment offered conflicting results for the four diseases under study. The grouping of electrically-related job titles (method #1) as the means of identifying individuals having probable occupational magnetic field exposure has been used in several studies.[13,54,64] One of the difficulties with this method is the inherent misclassification of individuals with other occupations that may have moderate or even high magnetic field exposure. The use of multiple exposure levels based on job title and industry (method #2) helped to capture some of the individuals classified as unexposed when only using the grouping of electrical occupations. The introduction of the JEM (method #3) suggested, however, that there may be additional misclassification of non-electrical occupations such as mechanics.

Previous reports suggested an association between amyotrophic lateral sclerosis and occupations with potential magnetic field exposure. In a clinic-based study of amyotrophic lateral sclerosis patients, the odds ratios were 7.5 and 5.5 among the upper quartiles of magnetic field exposure for total occupational exposure and average occupational exposure, respectively.[15] A review of occupational data from five electric utilities found a significant association between magnetic fields and amyotrophic lateral sclerosis deaths, particularly when looking at exposures of long duration.[14] Both of these recent studies utilized magnetic field exposure assessments specific to job title and duration of employment. Three other case-control studies have also identified electrical occupations as a risk factor for amyotrophic lateral sclerosis.[13,16,44]

Individuals with electrical occupations, however, may have greater potential for electric shock events. Three case-control studies have reported history of electrical shock as a specific trauma associated with amyotrophic lateral sclerosis,[16,42,184] although others have failed to support this relationship.[40] In our study, the moderate risk for electrical occupations disappeared when individuals were assigned to quartile of estimated magnetic field values. These findings support the hypothesis that electric shock or another unidentified variable associated with electrical occupations, rather than magnetic field exposure, were responsible for the observed associations with amyotrophic lateral sclerosis.

Our findings do not support an association between occupational magnetic field exposure and Alzheimer's disease when classifying exposure by electrical work (method #1) or the JEM (method #3). Risk estimates for this association in a recent series of studies ranged from 2.9 to 3.9, and were particularly influenced by sewing occupations.[9,10] We were unable to corroborate an association with sewing machine work in this analysis since there were inadequate numbers of men with these job titles in our study population. A Swedish study with more quantitative exposure assessment found a higher risk (odds ratios 2.4-2.7) for Alzheimer's disease among subjects whose most recent job had an average magnetic field exposure of more than 2 milliGauss.[17] Schulte et al.[11] suggested an association between occupations with probable magnetic field exposure and Alzheimer's disease mortality based on death certificates in over 27 states from 1982-1991. Although not hypothesized *a priori*, the study found an occupational clustering of electrical workers with Alzheimer's disease, particularly for electricians, power transmitter installers, electrical and electronic technicians, broadcast

operators, and electrical and electronic engineers.[12] Savitz et al.[13] reported a slightly elevated risk for Alzheimer's disease among individuals with electrical occupations (odds ratio and 95% confidence interval = 1.2 (1.0, 1.4)) with the strongest association observed among electricians and power plant operators. Our study, with approximately one-fifth the number of exposed cases, did not have adequate power to detect an elevation in risk of this magnitude. Finally, a review of Alzheimer's disease deaths among a large cohort of electric utility workers demonstrated a modest, but imprecise, rate ratio of 2.1 for individuals with magnetic field-exposed jobs for more than 20 years.[14]

The Parkinson's disease case group was intended to be a negative comparison analysis to validate our methods. Unlike previous studies,[13,14] we found a positive association between Parkinson's disease and history of electrical and other magnetic field-related occupations. In addition to electricians and electrical/electronic engineers, welders were over-represented among cases. Welders are exposed to high levels of magnetic fields, as well as other potentially neurotoxic agents such as metals which have been implicated as putative risk factors for Parkinson's disease.[50,185] In one study, based on few observations, all cases reporting aluminum exposure had been welders.[185]

The inclusion of a brain cancer case-control group in this investigation was also intended to validate the methodology, since most case-control studies based on United States death certificate data have demonstrated a relationship between brain cancer and occupations with high magnetic field exposures (odds ratios 1.4-3.9).[60-62,64] The only death certificate study reporting a lack of association between magnetic field-related occupations and brain cancer was based on an industry grouping of communication and

utilities rather than specific job titles.[58] We were unable to replicate the findings from other death certificate based case-control studies of brain cancer by categorizing potential magnetic field exposure according to job title and industry (methods #1 and #2). When exposure was assigned by JEM geometric mean (method #3), there was suggestion of a slight, imprecise elevation in risk for the highest quartile.

The primary limitation of this study was the inherent inaccuracy associated with the use of death certificates for collecting disease and exposure data. This is particularly true when considering neurological disorders with complex case definitions and a high potential for misdiagnosis. Among the four diseases considered in this study, amyotrophic lateral sclerosis and brain cancer have been found to be accurately reported in at least 87% of cases.[2,41,186-188] In contrast, Alzheimer's disease and Parkinson's disease are disorders of long duration, and patients are likely to die of unrelated primary causes. The reporting accuracy for these two diseases was shown to be 60 to 65% for any mention on death certificates, the selection criteria used in this study.[5,189,190]

Since these diseases were associated with social class and race in this population (Table 3.2), it is possible that the inaccuracies in the reporting of these diseases on death certificates may have been differentially misclassified with respect to our exposure of interest. Based on the distribution of electrical occupations and the JEM estimates of geometric mean by occupational grouping, the category of service/labor and others had higher magnetic field exposures. Professional and managerial workers, who are less likely to have magnetic field exposure, were more likely to have these diseases listed on their death certificates. This relationship was in agreement with previous studies showing "white-collar" and professional workers to be at increased risk for brain

cancer[55,191] but contrary to reports indicating that higher education and socio-economic status was protective for Alzheimer's disease.[192-195] This contradiction suggested that the elevated reporting of these diseases for the professional and managerial occupations in our population may reflect, in part, diagnostic sensitivity bias rather than true risk. Thus, we cannot rule out the possibility that the odds ratios presented in Table 3.3 underestimate the risk between these diseases and occupational magnetic field exposure.

The validity of this study also was dependent upon the accurate recording of occupational data on death certificates. Occupations reported on death certificates have been found to be in agreement with next-of-kin interview or employment records 48% to 76% of the time.[196-200] White males, comprising over 97% of our study population, had the highest rate of accuracy for occupational reporting.[197] In a study of brain cancer and magnetic field exposure in utility workers, accuracy of death certificate coding for exposed occupations was low overall but similar to unexposed occupations; concordance rates ranged from 54% to 80% for lineman, electricians, power plant operators, mechanics, and welders.[200] Exposure misclassification due to the use of death certificates versus known job history resulted in a slight bias in the risk estimate toward the null.[200] Reporting accuracy of industry on death certificates, a variable used in one of our exposure assessment methods, ranged from 67% to 75%.[197,198,200] However, death certificate reporting of job titles and industry is restricted to the subject's usual occupation and is limited by the lack of data for duration of employment.

Electrical work has often been used as a proxy in epidemiological investigations for exposure to electric and magnetic fields. However, this grouping of occupations is not specific with regard to any particular exposure of interest. By contrast, the JEM used in the current study was specific for estimated magnetic field exposures by job title for electrical and non-electrical occupations. Among the methods evaluated, the JEM fit best with our *a priori* hypothesis that an association exists between occupational magnetic field exposure and brain cancer, but not with Parkinson's disase. The findings for brain cancer were of limited strength and precision, although the risk estimate fell into the range of a recently published meta-analysis.[8] We were unable to replicate previous findings of an association between occupational magnetic field exposure and Alzheimer's disease or amyotrophic lateral sclerosis using the JEM. This conclusion must be qualified, however, due to the inherent inaccuracies associated with death certificate data, and the potential for differential misclassification of neurodegenerative disease reporting with respect to occupation.

Table 3.1. Descriptive data for male cases and controls of Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and brain cancer studies in Colorado, 1987-1996.

Attribute	Alzheimer's disease		Amyotrophic lateral sclerosis		Parkinson's disease		Brain cancer	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Number	1556	1556	312	1248	1477	1477	853	853
Age at death								
30-39	--	--	7	28	1	1	73	73
40-49	--	--	21	84	1	1	131	131
50-59	--	--	43	172	10	10	172	172
60-69	80	80	95	380	80	80	211	211
70-79	489	489	98	392	553	553	187	187
80 +	987	987	48	192	832	832	79	79
Race								
Non-Hispanic white	1532	1504	309	1197	1455	1441	834	797
Other	24	52	3	51	22	36	19	56
Education*								
≥ 12 years	797	740	200	715	800	716	582	528
< 12 years	445	483	61	310	398	475	125	172
Year of death								
1987-1991	717	717	148	592	647	647	376	376
1992-1996	839	839	164	656	830	830	477	477
Occupational grouping**								
Managerial/professional	410	368	114	283	405	358	311	216
Technical/sales	295	256	61	227	297	240	137	145
Service/labor	851	932	137	738	775	879	405	492

* There are no educational data available for all 1987-88 records.

** Occupations are grouped into the following broad categories: 1 – managerial/professional; 2 – technical/sales/administrative support; 3 – service/farming/craft/repair/operators/ fabricators/laborers.

Table 3.2. Risk estimates (odds ratios and 95% confidence intervals) for each case-control group by race, education, and occupational grouping.

Attribute	Alzheimer's disease	Amyotrophic lateral sclerosis	Parkinson's disease	Brain cancer
Race				
Non-Hispanic white vs. other	2.21 (1.35, 3.60)	4.39 (1.36, 14.2)	1.65 (0.97, 2.82)	3.08 (1.82, 5.24)
Education *				
≥ 12 years vs. < 12 years	1.17 (0.99, 1.38)	1.42 (1.04, 1.95)	1.33 (1.13, 1.58)	1.52 (1.17, 1.97)
Occupational grouping **				
Managerial/professional	1.22 (1.03, 1.44)	2.17 (1.63, 2.88)	1.28 (1.08, 1.52)	1.75 (1.41, 2.18)
Technical/sales	1.26 (1.04, 1.53)	1.45 (1.03, 2.03)	1.40 (1.15, 1.71)	1.15 (0.88, 1.50)
Service/labor (Referent)	1.00	1.00	1.00	1.00

* There are no educational data available for all 1987-88 records.

** Occupations are grouped into the following broad categories: 1 – managerial/professional; 2 – technical/ sales/administrative support; 3 – service/farming/craft/repair/operators/ fabricators/laborers.

Table 3.3. Adjusted odds ratios for each case-control group using three methods of exposure assessment.*

	Alzheimer's disease		Amyotrophic lateral sclerosis		Parkinson's disease		Brain cancer	
	Cases	Adj. OR (95% CI)	Cases	Adj. OR (95% CI)	Cases	Adj. OR (95% CI)	Cases	Adj. OR (95% CI)
Method #1 †								
Electrical	53	1.05 (0.71, 1.56)	19	2.30 (1.29, 4.09)	47	1.55 (0.98, 2.45)	40	1.05 (0.66, 1.67)
Non-electrical (Referent)	1503	1.00	293	1.00	1430	1.00	813	1.00
Method #2 ‡								
Definite/Probable MF exposure	62	1.21 (0.83, 1.76)	19	1.75 (1.00, 3.06)	64	1.76 (1.17, 2.65)	46	0.98 (0.64, 1.51)
Possible MF exposure	285	1.15 (0.95, 1.39)	51	1.18 (0.83, 1.67)	272	1.17 (0.96, 1.42)	139	1.19 (0.91, 1.56)
No MF exposure (Referent)	1209	1.00	242	1.00	1141	1.00	668	1.00
Method #3 §								
1 (Highest Quartile)	330	0.84 (0.65, 1.09)	68	1.02 (0.66, 1.58)	304	1.14 (0.87, 1.48)	172	1.32 (0.92, 1.89)
2	352	0.97 (0.75, 1.24)	54	0.78 (0.50, 1.21)	380	1.21 (0.94, 1.54)	171	0.94 (0.67, 1.31)
3	406	0.97 (0.76, 1.25)	75	0.82 (0.54, 1.23)	359	1.10 (0.85, 1.41)	193	1.06 (0.76, 1.48)
4 (Referent)	218	1.00	59	1.00	202	1.00	138	1.00

Adj. OR = adjusted odds ratios; CI = confidence interval; MF = magnetic field

* All odds ratios adjusted for race (white/non-white) and social class (1 - managerial/professional; 2 - technical/sales/administrative support; 3 - service/farming/craft/repair/operators/ fabricators/laborers).

† Based on references [13,54,64].

‡ Based on reference [60].

§ Based on quartile of geometric mean exposure as assigned to job titles by the Job Exposure Matrix.

Table 3.4. Crude odds ratios for each case-control group using three methods of exposure assessment.*

	Alzheimer's disease		Amyotrophic lateral sclerosis		Parkinson's disease		Brain cancer	
	Cases	Adj. OR (95% CI)	Cases	Adj. OR (95% CI)	Cases	Adj. OR (95% CI)	Cases	Adj. OR (95% CI)
Method #1 †								
Electrical	53	1.06 (0.72, 1.57)	19	2.18 (1.23, 3.86)	47	1.48 (0.94, 2.34)	40	1.09 (0.69, 1.72)
Non-electrical (Referent)	1503	1.00	293	1.00	1430	1.00	813	1.00
Method #2 ‡								
Definite/Probable MF exposure	62	1.20 (0.82, 1.74)	19	1.64 (0.95, 2.84)	64	1.70 (1.13, 2.55)	46	1.07 (0.70, 1.63)
Possible MF exposure	285	1.09 (0.91, 1.31)	51	1.06 (0.76, 1.49)	272	1.09 (0.90, 1.31)	139	1.11 (0.85, 1.44)
No MF exposure (Referent)	1209	1.00	242	1.00	1141	1.00	668	1.00
Method #3 §								
1 (Highest Quartile)	330	0.81 (0.63, 1.03)	68	0.72 (0.48, 1.07)	304	1.04 (0.81, 1.34)	172	0.94 (0.68, 1.30)
2	352	0.94 (0.73, 1.20)	54	0.60 (0.40, 0.92)	380	1.16 (0.91, 1.48)	171	0.77 (0.56, 1.06)
3	406	0.89 (0.71, 1.13)	75	0.62 (0.42, 0.91)	359	0.97 (0.76, 1.23)	193	0.84 (0.62, 1.16)
4 (Referent)	218	1.00	59	1.00	202	1.00	138	1.00

† Based on references [13,54,64].

‡ Based on reference [60].

§ Based on quartile of geometric mean exposure as assigned to job titles by the Job Exposure Matrix.

Table 3.5. Job Exposure Matrix time weighted average geometric mean magnetic field values assigned to electrical occupations. *

BOC Code	Job Title	Geometric Mean (mG)	No. of observations
055	Electrical and electronic engineers	1.9	67
213	Electrical and electronic technicians	2.6	29
228	Broadcast equipment operators	--†	--
523	Electronic repair, communication, and industrial equipment	2.4	57
525	Data processing equipment repairers	0.9	1
526	Household appliance and power tool repairers	2.3	32
527	Telephone line installers and repairers	1.7	32
529	Telephone installers and repairers	1.4	10
533	Miscellaneous electrical and electronic equipment repairers	4.7	4
555	Supervisors, electricians, and power installers and repairers	--†	--
575	Electricians	5.5	56
576	Electrician apprentices	5.5	56
577	Electric power installers and repairers	9.4	150
695	Power plant operators	7.8	51
773	Motion picture projectionists	6.3	14

BOC Code = Occupations Codes from the 1980 United States Bureau of Census Classified Index of Occupations and Industry; mG = milliGauss.

* Touchstone J, Yost M, unpublished data, 1999.

† The job exposure matrix used in this study does not contain geometric mean data for these job titles.

Table 3.6. Job titles with the highest geometric means based on Job Exposure Matrix.*

BOC Code	Job Title(s)	Geometric Mean (mG)	No. of observations
744	Textile sewing machine operators	29.9	2
783	Welders and cutters	9.5	42
577	Electrical power installers and repairers	9.4	150
695	Power plant operators	7.8	51
666-668, 673-674	Textile, apparel, and furnishings operators and tenders	7.8	5
496	Timber cutting and logging operations	7.6	9
773	Motion picture projectionists	6.3	14
197	Public relations specialists	5.6	1
575-576	Electricians and electrician apprentices	5.5	56
669	Shoe repairers	4.9	4
518, 533	Industrial machinery and electrical/ electronic equipment repariers	4.7	4
646, 653, 654	Sheet metal and lay-out workers	3.9	12
823, 825, 826	Railroad conductors and operators	3.9	7
547, 549	Mechanics and repairers	3.5	7
346, 354	Mail and paper handling machine operators; postal clerks	3.1	10

BOC Code = Occupations Codes from the 1980 United States Bureau of Census Classified Index of Occupations and Industry; mG = milliGauss.
* Touchstone J, Yost M, unpublished data, 1999.

CHAPTER 4

ASSESSMENT OF AMYLOID BETA PROTEIN IN ELECTRIC UTILITY WORKERS EXPOSED TO MAGNETIC FIELDS

4.1 Introduction

Alzheimer's disease is a progressive neurodegenerative disease of poorly understood etiology. Recent epidemiological studies have suggested a relationship between occupational magnetic field exposures and Alzheimer's disease,[9-11,13,14,17] yet the biological relationship whereby this could occur is unclear. Magnetic fields have been putatively associated with a reduced biosynthesis of the indoleamine hormone melatonin in humans.[157-159,201] Perturbations in melatonin concentrations could be related to the expression of the amyloid beta protein (Abeta),[165,166] a 40-42 amino acid peptide derived from the transmembrane amyloid precursor protein found in neuronal cells and circulating lymphocytes and platelets.[76,77,202] Abeta is the major component of the extracellular deposits found in both the cerebral neuropil and the cerebrovasculature of Alzheimer's patients. Soluble forms of Abeta(1-40) and Abeta(1-42) have been isolated from human plasma,[93,95,96,102] suggesting a role for circulating Abeta in the cerebrovascular and, to a lesser extent, the cerebral amyloid lesions associated with Alzheimer's disease. Elevated levels of soluble Abeta in plasma have been observed among pre-symptomatic carriers of familial Alzheimer's mutations and individuals with

Down's syndrome who generally express the pathological lesions of Alzheimer's disease by the fourth decade of life.[89,101-103]

Electric utility workers have high magnetic field exposures, and suppression of the urinary melatonin metabolite, 6-hydroxymelatonin sulfate (6-OHMS), has been observed in this occupational group.[157,158,203] The purpose of this study was to examine if magnetic field exposures, 6-OHMS concentrations, or certain personal characteristics are associated with the peripheral expression of free soluble Abeta among a similar population of electric utility workers.

4.2 Methods

Male employees from three electric utilities participated four times over the course of one year in a longitudinal study of magnetic field exposure and urinary excretion of 6-OHMS. The utilities, all located in Northern Colorado, were the City of Fort Collins Division of Light and Power, the Platte River Power Authority, and the Poudre Valley Rural Electric Authority. Subjects were asked to participate in the study of magnetic fields, melatonin, and Abeta during one of their weeks of participation. After a description of the objectives and methods of the study, informed consent was obtained from each individual willing to participate (see Appendix C). Of the 70 subjects that were approached, 61 (87%) agreed and were available to participate in this study (see Appendix D for power calculations).

Individuals were asked to wear a personal data logging device, the EMDEX II meter (Enertech Consultants, Campbell, CA), for magnetic field measurements. Meters were calibrated quantitatively every three months and functionally calibrated before and

after each use. The meters stored broadband magnetic field measurements every 15 seconds and were worn in a pouch around the waist, both at work and at home, for the first three days of an individual's work week. During their four days of participation, each individual recorded the times they went to bed, woke up, left for work, arrived at work, left work, and arrived at home (see Appendix E).

On the morning of the fourth work day the EMDEX II meters were retrieved, and participants were asked to complete a questionnaire (see Appendix F). The questionnaire included information on work activities, current medical conditions and use of pharmaceuticals, family history of neurodegenerative conditions, history of head injury, exposure to aluminum, and lifestyle factors such as smoking, alcohol intake, dietary information, and use of vitamin supplements. These data were combined in a computerized database with descriptive data from a questionnaire the subjects had completed in a previous study.

All magnetic field data were downloaded to a personal computer using EMDEX version 2.11 software (Enertech Consultants, Campbell, CA). We used MFLTCALC software (Colorado State University, Fort Collins, CO) to generate magnetic field exposure metrics for each individual's work hours. The exposure metrics are arithmetic time weighted average (TWA), geometric TWA, cumulative exposure, cumulative exposure over 2 milliGauss (mG), rate of change metric (RCM), and standardized rate of change metric (RCMS). RCM is an indication of variability and auto-correlation between successive 15 second magnetic field measurements, and RCMS is the ratio of RCM and the standard deviation.[204] Low RCMS values are indicative of temporally stable magnetic field exposures.

$$\text{RCM (milliGauss/15 seconds)} = \sqrt{\frac{\sum (MF_1 - MF_2)^2}{(n-1)}},$$

where MF1 and MF2 are successive 15 second magnetic field measurements and n is the number of measurements within a given exposure period.[204]

RCMS (per 15 seconds) = RCM / SD,

where SD is the standard deviation of the magnetic field measurements in a given exposure period.[204]

One blood sample from each subject was collected by a phlebotomist in a 5 milliliter tube containing Na-EDTA. All samples were held for approximately the same period of time prior to processing and storage, and no additional preservative was added (see Appendix G). The holding time was determined by the maximum time required for transportation from the furthest field collection site to the laboratory. All blood samples were taken at the subjects' place of work between 2:50 p.m. and 4:50 p.m. on the third work day of their week of participation. Samples were transported on ice to Colorado State University and centrifuged for 10 minutes at 3000 rotations per minute in 4°C using a Sorvall RC-58 (DuPont). Plasma was separated and stored in polypropylene tubes at -85°C for later analysis. All plasma samples were centrifuged within 65 to 85 minutes following the blood draw and placed in the freezer within 75 to 115 minutes following the blood draw.

The Abeta concentrations for this study were determined using a double antibody sandwich ELISA method developed by Mehta el al.[102] The monoclonal antibody 6E10 (specific to an epitope present on 1-16 amino acid residues of Abeta) and biotinylated polyclonal antibodies R162 and R165, raised against Abeta(32-40) and Abeta(33-42)

respectively, were donated from the New York State Institute for Basic Research, Department of Immunology. This method allowed the quantification of both Abeta(1-40) and the more aggregable form of the protein, Abeta(1-42). Each plasma sample was assayed in triplicate on two plates, one using R162 and one using R165. The ELISA procedure has been described elsewhere[102] and is briefly outlined below (see Appendix I for a more detailed description of the procedure and solutions used).

All 96-well microtiter ELISA plates were coated with the monoclonal antibody at the same time. One hundred microliters of 6E10 (2.5 ul/mg) diluted in carbonate-bicarbonate buffer at pH 9.6 were coated on U96 Maxisorp Immunoplates (NUNC, Denmark) and incubated at 4°C overnight. Plates were washed the next day with 10mM phosphate buffered saline, 0.85% NaCl, containing 0.05% of Tween-20 (PBST), and then stored at -20°C until the time of use.

After thawing, plates were blocked for 1 hour at room temperature using 200 ul of PBST with 1% bovine serum albumin (BSA) (Sigma, St. Louis, MO). After washing with PBST, 100 ul of standards (Abeta(1-40) and Abeta(1-42): Bachem, Torrance, CA) diluted in PBST + 1% BSA or 100 ul of undiluted plasma sample was applied and incubated for 2 hours at room temperature, then at 4°C overnight. The standards ranged from 39.06 pg/ml to 625 pg/ml and from 9.77 pg/ml to 1250 pg/ml for Abeta(1-40) and Abeta(1-42), respectively. The following day, plates were washed and incubated with R162 and R165 diluted 1:400 in PBST + 1% BSA for 1.5 hours at room temperature. After washing again, neutravidin-horseradish peroxidase conjugated (Pierce, Rockford, IL) diluted 1:10,000 in PBST was applied and incubated for an additional 1 hour at room temperature. Following incubation, plates were washed and 100 ul of a 1:1 mixture of

tetramethylbenzidine (peroxidase substrate) and hydrogen peroxide was added, using TMB Microwell Peroxidase Substrate system (Kirkegaard and Perry Lab, Gaithersberg, MD). The reaction was stopped after 25 minutes by adding 100 ul of 1 M phosphoric acid. The optical density (OD) was measured at 450 nm using a MR5000 microplate reader (Dynatech). The plasma sample concentrations of Abeta(1-40) and Abeta(1-42) were calculated from the standard curve for each plate. The relationship between OD and Abeta standards was determined by a third order polynomial to a log-linear scale using Cricket 1.3 graphing software.

Subjects were also asked to provide total overnight urine samples and post-work urine samples during their week of participation. All urine samples were collected in labeled containers and placed in refrigerators located at their place of work. Subjects were asked to record the sample number and date of each sample on a sample log form (see Appendix E). Urine samples were retrieved, transported on ice to Colorado State University, aliquoted to polypropylene tubes, and stored at -20°C for later analysis. The melatonin metabolite, 6-OHMS, was measured in urine by radioimmunoassay at the Animal Reproductive and Biotechnology Laboratory at Colorado State University using materials purchased from CIDTech (Mississauga, Ontario, Canada). In order to adjust for dilution of urine, creatinine concentrations were also determined using a Beckman Creatinine Analyzer II (Brea, CA).

All data, including magnetic field exposure metrics, 6-OHMS results, questionnaire responses, and Abeta parameters, were combined in a database using Statistical Application Software version 6.12 (Cary, NC). Questionnaire variables were screened for inclusion in the statistical model using regression for continuous variables

and analysis of variance for categorical variables. The association between Abeta variables and magnetic field exposure metrics and 6-OHMS variables was originally assessed by correlation analysis. Magnetic field and 6-OHMS variables were then divided into tertiles and included in an analysis of variance model using Proc GLM. The dependent Abeta variables were square-root transformed to satisfy assumptions of normality and homogeneity of variance (see Appendix J).

4.3 Results

There was no marked difference in the demographic characteristics of those who participated in this study and those who refused or were unable to participate. The mean age and range of ages among the 61 participants and the 9 non-participants was 41 years (22 to 54) and 43 years (26 to 53), respectively. Based on a t-test, the mean age of these groups was not statistically different ($p = 0.43$). The majority of individuals classified themselves as non-Hispanic white, 87% and 89% for participants and non-participants, respectively. The two groups did not differ by categorical groupings of job title. Most of the participants were classified as electrical workers directly involved in electric power distribution or generation, and all administration and maintenance workers were categorized as comparison workers. For both the participant and the non-participant groups, 67% of the workers were classified as distribution and generation workers. One individual was excluded from the study due to a malfunction in the EMDEX meter. The racial and age distribution among the remaining 60 participants are presented in Tables 4.1 and 4.2, respectively.

The magnetic field exposure metrics for the study population during their work period on the day of the blood draw are summarized in Table 4.3. In general, the intensity of magnetic field exposure was higher among distribution and generation workers compared to administration and maintenance workers. RCM was also higher among distribution and generation workers, but there was no difference in RCMS between the two groups. Similar results were observed for magnetic field measurements averaged over the two hours prior to the blood draw and over three work days (see Appendix K).

Descriptive statistics of the Abeta results for the participant's blood samples are presented in Table 4.4. The Abeta results for our study population were consistent with previously reported values which ranged from detection levels to 242 pg/ml for Abeta(1-40) and as high as 887 pg/ml for Abeta(1-42) in a population of similar age distribution.[102] Based on a control sample that was repeated on every plate, the intra-plate coefficient of variation ranged from 4% to 24%, and the inter-plate coefficient of variation was 16% and 36% for Abeta(1-40) and Abeta(1-42), respectively. Given this high inter-plate variation, particularly for the Abeta(1-42), all results were normalized to the value of this control sample on each plate. Five of the sixty samples had Abeta(1-42) values below the lowest standard. After confirming these in a separate assay, all five samples were assigned an Abeta(1-42) value of one-half the lowest standard, or 4.9 pg/ml.

Crude and adjusted Abeta concentrations for personal demographic, behavioral, and activity variables are presented in Table 4.5. Subjects indicating that they had engaged in physical work on the day of the blood draw had lower levels of Abeta,

particularly Abeta(1-42). Adjusted least-squares means of the Abeta parameters for individuals who had engaged in at least 2 hours of physical work versus individuals with no physical work were reduced by 16% ($p = 0.059$), 43% ($p = 0.041$), and 40% ($p = 0.051$) for Abeta(1-40), Abeta(1-42), and the Abeta ratio, respectively (see also Appendix L). Individuals reporting an annual income of less than $30,000 had higher levels of Abeta than those reporting higher incomes ($p < 0.05$ for Abeta(1-40) and Abeta(1-42)). There was a strong positive association between consumption of vegetables and each of the Abeta parameters ($p < 0.01$ for all three dependent variables). When analyzed as a continuous variable, age showed a positive assocation with Abeta(1-40) after adjusting for income, physical work, and vegetable consumption ($p = 0.01$), but no association with the other Abeta parameters. All adjusted models presented below included age, income, physical work, and vegetable consumption. No other questionnaire variables had statistically significant associations with any of the Abeta parameters. None of the variables relating to potential aluminum exposure (use of antacids, antiperspirants, or aluminum cookware) were associated with Abeta. There also was no association between reported head injury, fruit consumption, or use of vitamin supplements and concentrations of Abeta in blood.

Using Spearman rank correlation, there was no association between measures of workplace magnetic field intensity and the Abeta variables. This remained true for magnetic field measurements averaged over the three work days and the two hours prior to blood draw (see Appendix M). There was no correlation between RCMS and Abeta, but RCM was inversely associated with Abeta(1-42) and the ratio of Abeta(1-42) to Abeta(1-40), yielding correlation coefficients of –0.21 ($p = 0.11$) and –0.27 ($p = 0.04$),

respectively. The distribution of individuals by tertile of RCM and tertile of the Abeta parameters showed a greater number of individuals with high RCM exposure and low Abeta than would be expected by chance (Fisher's overall exact Chi-Square, p = 0.023, 0.188, and 0.066 for Abeta(1-40), Abeta(1-42), and the Abeta ratio, respectively). The adjusted least-squares means of Abeta(1-42), but not Abeta(1-40), were inversely related to tertile of RCM. with means of 294 pg/ml and 192 pg/ml for the lowest and highest tertiles, respectively (p = 0.050). The adjusted least-squares means of the Abeta Ratio also demonstrated a trend by tertile of RCM values with ratios of 1.90 and 1.08 for the lowest and highest tertiles, respectively (p = 0.01) (see Appendix N).

Since the RCM metric is a combination measure of variability and auto-correlation, we further assessed the association between Abeta and variability in magnetic field exposures by using the standard deviation of individuals' magnetic field means as a predictive variable. Similar to the findings for RCM, Abeta(1-40) was not associated with the standard deviation of the mean magnetic field exposures. The adjusted least-squares means for Abeta(1-42) were 323 pg/ml and 212 pg/ml for the lowest and highest tertiles of the standard deviation (p = 0.078), and the Abeta ratio had a robust inverse relationship (p = 0.022) (see Appendix N).

To explore the possibility that variability in magnetic field exposure may have been an indicator for physical activity at work, we evaluated RCM and standard deviation of the mean after stratification by the physical work variable. Figure 4.1 indicates that there is still some indication of a decreasing trend in the Abeta ratio by tertile of RCM among those indicating that they did not engage in physical work, but the association is not as strong as that found among those engaged in physical work. The inverse

association between standard deviation of the mean and the Abeta ratio was not observed among those indicating no physical work on the day of the blood draw (see Figure 4.2).

As expected, diurnal variation was observed between the nocturnal, or overnight, and afternoon, or post-workshift, 6-OHMS concentrations. The mean (± SEM) of overnight creatinine-adjusted 6-OHMS (6-OHMS/cr) concentrations was five times higher than post-workshift 6-OHMS/cr (22.5 ± 2.1 and 4.0 ± 1.0, respectively) (see Appendix O). When analyzing exposures for the work day of the blood draw, we did not observe an association between magnetic fields and post-workshift 6-OHMS/cr (see Appendix P). However, post-workshift 6-OHMS/cr was inversely associated with Abeta, yielding a Spearman correlation coefficient of –0.22 ($p = 0.08$) and –0.21 ($p = 0.10$) for Abeta(1-42) and the Abeta ratio, respectively (see Appendix Q). The adjusted least-squares means of Abeta(1-42) and the Abeta Ratio demonstrated a decreasing, yet not statistically significant, trend by tertile of post-workshift 6-OHMS/cr, but this trend was not evident for Abeta(1-40) (see Appendix Q). Unfortunately, the timing for collection of some blood and post-workshift urine samples was not consistent, as certain individuals continued to work into the late afternoon and early evening after the blood draw. In order to address this problem, we conducted the analysis using exclusion criteria based on the difference between the time of an individual's blood draw and the end of his workshift on that day. As shown in Table 4.6, the absolute magnitude, and, to some degree, the statistical significance of the inverse relationship between post-workshift 6-OHMS/cr and the Abeta ratio grew stronger as the blood and urine samples included in the analysis were temporally closer.

4.4 Discussion

Several studies have examined the genetic factors associated with heightened expression of Abeta,[89,101-103] yet there has been no description of environmental or physiological factors that may influence this parameter. We did not observe any correlation between personal measures of magnetic field intensity and the Abeta parameters. This lack of assocation remained true for intensity measures averaged over different time periods: work exposure on the day of the blood draw, work exposure over the three work days prior to the blood draw, and work exposure for two hours prior to the blood draw. One of the limitations of this study was the low level of exposure to magnetic fields relative to previous studies of highly exposed workers. The time weighted average exposure for this study population was approximately 3.0 mG with only slightly higher exposures among electric power distribution and generation workers. Other studies have reported magnetic field exposures of approximately 10 mG, 6 mG, and 13 mG for lineworkers, generation workers, and substation operators, respectively.[205] Feychting et al.[17] found an elevated risk of Alzheimer's disease among individuals whose previous occupation had an average magnetic field exposure of greater than 2.0 mG. By contrast, the elevated risks observed by Sobel et al.[9,10] were largely dependent upon sewing machine operators and similar occupations with magnetic field exposures close to 30 mG. Thus, although we observed differences in measures of magnetic field intensity between distribution and generation workers versus administration and maintenance workers, the exposures may not have been high enough to elicit a biological effect. Indeed, we did not observe an association between our measures of magnetic field exposure and 6-OHMS concentrations which was one

hypothesized mechanism whereby magnetic fields may perturb Abeta processing. It should be noted, however, that this finding was based on only one day of measured 6-OHMS, whereas previous studies have used a repeated measures design.[157-159]

The association between Abeta and measures of magnetic field intermittence (RCM) and temporal stability (RCMS) were incompatible with previously observed biological effects. Laboratory studies of different cell types suggested that magnetic field-induced increase in ornithine decarboxylase enzymatic activity was dependent upon temporally stable fields, and the superposition of temporally incoherent magnetic fields inhibits these effects.[206,207] Previous findings of reduced post-workshift and nocturnal 6-OHMS/cr concentrations among electric utility workers were also dependent, in part, upon exposure to temporally stable, or low RCMS, exposures.[157,158,203] Thus, if there were an association between magnetic field temporal characteristics and Abeta, we would expect an inverse relationship with the RCMS metric. By contrast, we found measures of magnetic field variability, RCM and the standard deviation of the mean, to be inversely associated with Abeta in plasma, particularly Abeta(1-42) and the ratio of Abeta(1-42) to Abeta(1-40). Given the lack of evidence for highly variable magnetic fields in eliciting biological effects, we were unable conclude that intermittent, or highly variable, magnetic field exposure affects Abeta processing or expression. One alternative explanation was that measures of magnetic field variability are surrogates for individual movement or physical activity, a variable found to be inversely correlated with Abeta. Stratified analysis of RCM and Abeta by the physical work variable suggested that intermittent magnetic field exposure remains inversely correlated with the Abeta ratio among those not engaged in physical work, yet to a lesser degree than for those indicating at least

some physical work. Without better measures of physical activity or a clearer understanding of the relationship between this variable and Abeta expression, however, we cannot rule out the possibility that highly variable magnetic fields may affect Abeta levels.

Regardless of whether or not magnetic fields diminished 6-OHMS levels in this study, we found some suggestion of a relationship between 6-OHMS and the Abeta ratio, particularly when excluding individuals whose urine sample was not taken simultaneouly with the blood draw. We did not observe an association between the previous evening's overnight 6-OHMS concentrations and Abeta levels, and with only one blood sampling opportunity in this study we were unable to assess the possibility that circulating Abeta levels follow a diurnal rhythm similar to melatonin. Measurement of melatonin and Abeta levels had not been investigated previously in humans, yet recent *in vitro* data provided support for this relationship. Neuroblastoma and pheochromocytoma cells demonstrated a sharp decline in the secretion of Abeta as well as the soluble form of the amyloid precursor protein following treatment with melatonin.[165,166]

There are at least two possibilities as to how melatonin may exert an effect on Abeta expression. First, melatonin is an endogenous protector against oxidative stress, both as a direct scavenger of hydroxyl radicals and as a stimulator of other antioxidant enzymes.[172-176] Conditions of oxidative stress introduced in cell culture have been shown to stimulate the release of both Abeta and the amyloid precursor protein.[178-180] Thus, given the potential for oxidative stress to moderate the processing of Abeta, melatonin may be an important factor in this relationship. It must be noted, however, that we did not observe any association with Abeta and smoking status, nor did we observe an

inverse relationship with the consumption of antioxidant sources such as vitamins, vegetables, or fruits.

The second mechanism whereby melatonin could affect Abeta processing is through its ability to stimulate calcium pump activity.[171] In cell cultures, non-homeostatic levels of intracellular calcium promoted Abeta release, suggesting the involvement of a calcium-requiring protease in processing of the amyloid precursor protein.[134,135] Hypothetically, Abeta aggregates could self-promote the release of soluble Abeta through its ability to stimulate calcium ion influx and/or impair calcium extrusion,[167-169] and melatonin has been shown to prevent this Abeta-induced intracellular calcium increase.[170] In addition to stimulation of calcium pump activity, melatonin's antioxidant properties may also help to protect against oxidative injury to sulfhydryl groups in these membrane pumps.

An attempt was made to account for factors that might influence Abeta expression and/or clearance. Given the dearth of previous studies on non-genetic determinants of Abeta expression, it was difficult to anticipate all potential confounding factors. The questionnaire item on vegetable consumption was included as a potential confounder because of the suggested relationship between oxidative stress and Abeta processing.[178-180] Our findings were, in fact, contrary to our *a priori* hypothesis concerning this variable, and other potential sources of antioxidants such as use of supplemental vitamins and fruit consumption demonstrated no association with Abeta concentrations. The fact that high levels of vegetable consumption were positively associated with the Abeta variables may be an indication of the effect of dietary factors on the detection of free soluble Abeta. Diets high in vegetable content result in a reduction in blood cholesterol

levels.[208-210] This relationship is partly due to the displacement of foods high in saturated fat and cholesterol, but there is also a direct effect of vegetable consumption on lowering lipoprotein levels when the intake of others foods is held constant.[210] Plasma proteins are known to bind with Abeta,[211-213] and the addition of purified lipoproteins to free Abeta resulted in a 90% reduction in the ability to detect Abeta by immunoassay.[211] Thus, to the extent that vegetable consumption is inversely correlated with individuals' lipoprotein levels, the positive relationship observed between vegetable intake and Abeta may be due to less sequestration, and greater detection, of free Abeta. This relationship is difficult to support, however, without additional dietary information or lipoprotein analysis. Moreover, we would expect to find a similar association between fruit intake and Abeta, but this was not observed in our study population.

The inverse association between physical work and Abeta is consistent with previous epidemiological studies indicating that physical activity and exercise may be protective for Alzheimer's disease. In a seven year prospective study of an elderly population, moderate to severe physical activity at work was found to be protective against Alzheimer's disease (relative risk and 95% confidence interval = 0.20 (0.06 – 0.68)).[214] By informant interview, a case-control study found Alzheimer's patients more likely to have been physically underactive in both the recent and distant past, yielding odds ratios of 6.25 and 3.50, respectively.[215] One possible explanation of this protective effect against Alzheimer's disease is the ability of physical activity and exercise to enhance cerebral perfusion and measures of cognition.[216,217] Our study indicates that physical activity is associated with lower levels of circulating free Abeta. It is not clear whether physical activity may play a role in promoting non-amyloidogenic processing of

the APP or in accelerating the breakdown and clearance of the protein from the periphery.

The physiological significance of soluble blood-borne Abeta has not been fully elucidated. Several lines of evidence suggest that peripherally available Abeta is a relevant indicator for future risk of Alzheimer's disease. First, although blood-borne Abeta can be derived from circulating sources,[76,77] neuronally-derived Abeta has been shown to be cleared into the blood.[99,100] Thus, genetic or environmental influences on Abeta expression in either the blood or cerebral compartment is reflected in circulating levels. Second, individuals that are genetically predisposed to the formation of senile plaques, including individuals with Down's syndrome as well as carriers of the mutations associated with familial forms of Alzheimer's disease, exhibited elevated levels of circulating Abeta(1-42).[89,101-103] Third, passage of blood-borne Abeta across the blood brain barrier has been observed in animal models,[105-107] suggesting that circulating Abeta may contribute to senile plaque formation. This property may be consistent with the apolipoprotein E4 (apoE4) polymorphism risk factor for Alzheimer's disease, as Abeta bound to apoE isoforms demonstrated significant transport across the blood brain barrier only for apoE4 but not for apoE2 or apoE3.[110] Fourth, it has been suggested that circulating soluble Abeta contributes to cerebral amyloid angiopathy, present in most Alzheimer's cases and present consistently in Down's syndrome.[211,218,219] Soluble Abeta(1-40) has been shown to be the principle component of these cerebral amyloid deposits;[220] but, as with senile plaques, Abeta(1-42) appears to be responsible for initial aggregation of the amyloid core.[221,222] Finally, circulating soluble Abeta caused increased contraction to the vasoconstrictor endothelin-1 and diminished relaxation to the

vasodilator bradykinin in rat aorta and bovine cerebral arteries.[118,119] *In vivo*, arterial infusion of Abeta(1-40) in rodents resulted in decreased blood flow and increased vascular resistance.[120] These vasoactive properties suggest that peripherally available Abeta could play a role in cerebral hypoperfusion and, consequently, in Alzheimer's disease pathogenesis.

The primary limitation of this study was the use of a biological marker that is not directly linked with a clinically recognized condition. The present study was conducted in a population of healthy workers under the age of 55. This group would not be expected to harbor the pathological lesions associated with Alzheimer's disease that might affect the expression of soluble Abeta in the blood, including plaque formation and/or disruption of the blood brain barrier. Elevated levels of Abeta in this group due to environmental or physiological factors would not necessarily indicate early stages of the disease; rather they would be suggestive of a pattern of effect that, if continued, could contribute to the cerebrovascular lesions and potentially to the senile plaques associated with Alzheimer's disease.

In conclusion, results presented here do not provide strong support for a direct association between occupational magnetic field exposure and peripheral expression of Abeta. We did find an inverse relationship between measures of magnetic field variability and the Abeta parameters, yet we cannot rule out the possibility that this observation was an indication of the effect physical activity may have on Abeta. Our findings do offer limited support, however, for an inverse relationship between melatonin concentrations and detection of soluble Abeta in blood. Additional research that

incorporates serial measurements of both melatonin and Abeta are needed to confirm these results.

Table 4.1. Racial distribution of study participants.

Race	Distribution/ Generation workers (n = 44)	Administration/ maintenance workers (n = 16)	Total (n = 60)
White, non-Hispanic	37	15	52
Hispanic	5	0	5
Native American	1	0	1
African American	1	0	1
Other	0	1	1

Table 4.2. Age distribution of study participants and mean age.

Age Group	Distribution/ Generation workers (n = 44)	Administration/ Maintenance workers (n = 16)	Total (n = 60)
< 30 years old	4 (9%)	3 (18%)	7 (12%)
30 – 39 years	13 (30%)	5 (31%)	18 (30%)
40 – 49 years	19 (43%)	6 (38%)	25 (42%)
≥ 50 years	8 (18%)	2 (13%)	10 (17%)
Total	44 (100%)	16 (100%)	60 (100%)
Mean age (± SE)	42.0 (± 1.2)	38.8 (± 2.3)	41.1 (± 1.1)

Table 4.3. Mean magnetic field exposure metrics for work period on the day of the subjects' blood draw.

Exposure metric	Distribution/ Generation workers (n = 44)	Administration/ maintenance workers (n = 16)	Total (n = 60)
Arithmetic TWA (mG)	3.67	1.08 *	2.97
Geometric TWA (mG)	1.19	0.64	1.05
Cumulative Exposure (mG-hrs.)	32.04	8.40 *	25.74
Cumulative Exposure > 2 mG (mG-hrs.)	27.72	4.56 *	21.54
RCM (mG/15 seconds)	4.51	1.24	3.64
RCMS (per 15 seconds)	0.73	0.75	0.74

TWA = time weighted average; mG = milliGauss; RCM = rate of change metric; RCMS = standardized rate of change metric.
* $p < 0.05$ for T-test of the difference between group means. Exposure metrics (except RCMS) were log-transformed for T-test to satisfy assumption of normality.

Table 4.4. Descriptive statistics of Abeta parameters among study participants.

	Abeta(1-40) (pg/ml)	Abeta(1-42) (pg/ml)	Abeta Ratio Abeta(1-42)/Abeta(1-40)
Number of samples	60	60	60
Mean	137.4	209.5	1.34
Standard Deviation	46.9	193.2	0.99
Minimum value	68.1	4.9	0.04
Maximum value	294.8	902.7	4.89

Table 4.5. Mean Values of Abeta Parameters for selected variables.[a]

Variable	Abeta(1-40)		Abeta(1-42)		Abeta Ratio [Abeta(1-42)/Abeta(1-40)]	
	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)
Age group (years)						
20-29 (n = 7)	138.3	137.3 (109.4-168.4)	243.0	252.7 (129.9-416.1)	1.65	1.75 (1.00-2.70)
30-39 (n = 18)	126.3	139.6 (115.3-166.2)	139.7	157.0 (75.5-268.1)	1.01	1.00 (0.53-1.63)
40-49 (n = 25)	134.8	161.4 (132.8-192.7)	155.5	215.2 (109.2-356.8)	1.06	1.21 (0.64-1.96)
≥ 50 (n = 10)	142.3	169.6 (134.8-208.4)	182.8	259.5 (122.3-447.6)	1.20	1.47 (0.73-2.46)
Race						
Non-White or Hispanic (n = 8)	145.0	178.8 (143.8-217.9)	227.4	237.5 (150.6-344.5)	1.48	1.85 (0.98-2.99)
White (n = 52)	132.3	157.8 (137.1-180.1)	155.5	332.0 (172.1-544.3)	1.08	1.42 (0.92-2.02)
Occupational group						
Administrative/ maintenance (n = 16)	121.0	140.4 (117.3-165.4)	162.1	225.6 (122.1-360.2)	1.30	1.59 (0.95-2.38)
Distribution (n = 44)	139.2	174.0 (150.8-199.1)	162.6	260.2 (157.5-388.5)	1.04	1.38 (0.84-2.05)
Generation (n = 11)	137.1	163.6 (134.1-196.0)	173.2	259.5 (129.7-434.3)	1.14	1.46 (0.76-2.41)
Income ($/year)						
≤ $30,000 (n = 6)	162.0	199.5 (156.8-247.4)	274.6	390.7 (192.3-658.8)	1.62	2.02 (1.00-3.42)
> $30,000 (n = 54)	130.9	125.0 (113.4-137.2)	153.7	136.6 (94.65-186.2)	1.08	1.00 (0.73-1.30)
Education						
≤ 12 years (n = 17)	136.9	167.4 (142.1-194.9)	166.9	252.5 (146.1-387.3)	0.99	1.40 (0.83-2.12)
> 12 years (n = 42)	134.1	153.3 (130.9-177.7)	168.0	238.1 (142.1-359.1)	1.18	1.49 (0.94-2.18)
Smoking						
Yes (n = 9)	131.1	156.5 (128.1-188.0)	110.9	187.1 (87.4-324.0)	0.79	1.11 (0.55-1.87)
No (n = 51)	134.3	161.8 (138.8-186.6)	174.8	278.6 (176.4-403.6)	1.19	1.65 (1.08-2.33)

Table 4.5 (continued). Mean Values of Abeta Parameters for selected variables.[a]

Variable	Abeta(1-40)		Abeta(1-42)		Abeta Ratio [Abeta(1-42)/Abeta(1-40)]	
	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)
Body mass index						
≤ 26 kg/m^2 (n = 30)	133.6	156.8 (133.9-181.4)	164.1	232.3 (137.8-351.2)	1.12	1.40 (0.83-2.12)
> 26 kg/m^2 (n = 30)	134.1	163.6 (139.9-189.1)	164.4	263.4 (161.5-389.7)	1.13	1.53 (0.97-2.22)
Post-workshift 6-OHMS/cr (ng/mg cr)						
≤ 1.38 (n = 20)	132.5	160.5 (135.5-187.7)	176.9	262.8 (155.3-398.8)	1.26	1.60 (0.99-2.36)
1.38 - 3.3 (n = 20)	146.7	163.1 (138.3-189.9)	213.7	257.6 (153.3-389.3)	1.33	1.49 (0.90-2.19)
> 3.3 (n = 20)	123.0	153.0 (126.3-182.0)	110.9	197.7 (99.0-330.1)	0.83	1.19 (0.63-1.59)
Physical work on day of blood draw						
0 min. (n = 40)	140.5	174.1 (153.3-197.3)	188.9	311.3 (210.5-431.6)	1.25	1.76 (1.21-2.40)
30–120 min. (n=10)	131.8	160.5 (130.4-193.7)	177.2	264.6 (136.4-435.1)	1.28	1.64 (0.90-2.60)
180–360 min. (n=10)	110.8	146.3 (118.8-176.7)	74.0	176.1 (78.7-312.1)	0.60	1.05 (0.50-1.80)
Previous head injury						
Yes (n = 8)	129.0	154.5 (123.2-189.6)	193.2	269.9 (132.5-455.4)	1.35	1.64 (0.86-2.67)
No (n = 52)	134.6	161.3 (139.7-184.7)	160.0	242.4 (151.5-354.6)	1.10	1.43 (0.92-2.04)
Vegetable intake						
> 2 servings per day (n=24)	157.3	182.9 (156.4-211.5)	275.6	357.7 (230.0-513.4)	1.65	1.93 (1.25-2.75)
1 or fewer serving per day (n = 36)	119.5	138.8 (119.0-160.3)	105.9	157.3 (86.9-248.4)	0.84	1.07 (0.61-1.59)

Table 4.5 (continued). Mean Values of Abeta Parameters for selected variables.[a]

Variable	Abeta(1-40)		Abeta(1-42)		Abeta Ratio [Abeta(1-42)/Abeta(1-40)]	
	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)	Crude mean (pg/ml)	Adjusted mean[b] (pg/ml)
Fruit intake						
2 or more servings per day (n = 23)	137.4	153.5 (131.8-176.9)	169.0	217.0 (130.2-326.2)	1.13	1.32 (0.83-1.94)
1 or fewer serving per day (n = 37)	131.8	171.9 (145.9-199.9)	161.3	304.9 (186.9-451.6)	1.12	1.73 (1.08-2.53)
Use of vitamin supplements						
Yes (n = 32)	140.4	166.4 (141.6-193.2)	194.3	279.6 (170.0-416.2)	1.27	1.61 (1.00-2.36)
No (n = 28)	126.6	156.3 (134.3-179.8)	132.9	228.3 (138.5-340.8)	0.97	1.38 (0.87-2.00)
Use of antacids						
Yes (n = 6)	133.9	152.5 (115.6-194.9)	161.8	229.5 (85.4-443.5)	1.15	1.46 (0.61-2.67)
No (n = 52)	134.1	160.8 (139.2-183.9)	166.4	251.5 (159.5-364.0)	1.14	1.48 (0.97-2.11)
Use of antiperspirants						
Yes (n = 23)	140.4	161.0 (138.1-185.8)	210.8	269.6 (168.7-394.0)	2.15	1.58 (1.02-2.26)
No (n = 35)	130.0	158.8 (133.9-186.0)	139.2	220.2 (122.5-346.7)	1.01	1.34 (0.78-2.04)
Use of aluminum cookware						
Yes (n = 26)	134.8	164.4 (138.5-192.1)	165.9	252.2 (144.7-389.3)	1.14	1.46 (0.87-2.22)
No (n = 32)	133.5	158.0 (135.7-182.3)	165.9	242.4 (151.5-354.6)	1.13	1.49 (0.95-2.15)

[a] Variations in subject number are due to missing data for selected variables.

[b] 95% confidence intervals in parentheses. Results adjusted for age, physical work, income, and vegetable intake using Proc GLM

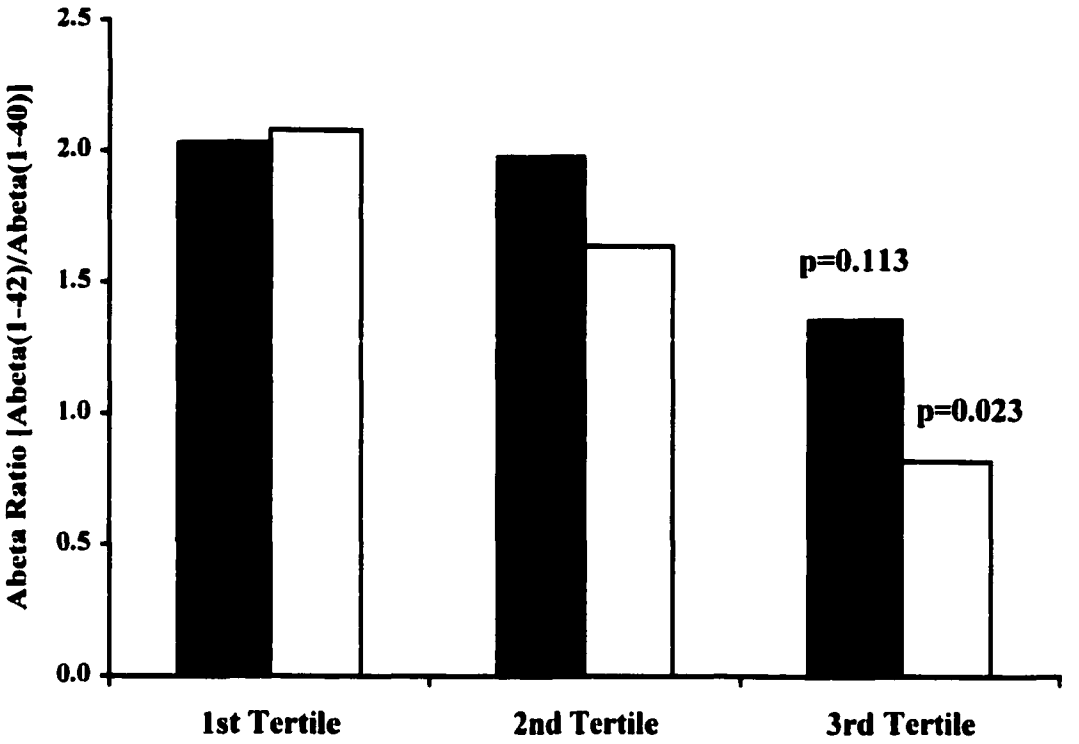

Figure 4.1 Abeta Ratio [Abeta(1-42)/Abeta(1-40)] Least-squares Means for workers with no (black bars) and some (white bars) physical work on the day of the blood draw by tertile of RCM, n = 60.[a]

[a] Means are adjusted for age, income, physical work, and vegetable consumption. p-values are for comparison of difference between means of 3rd and 1st tertiles.

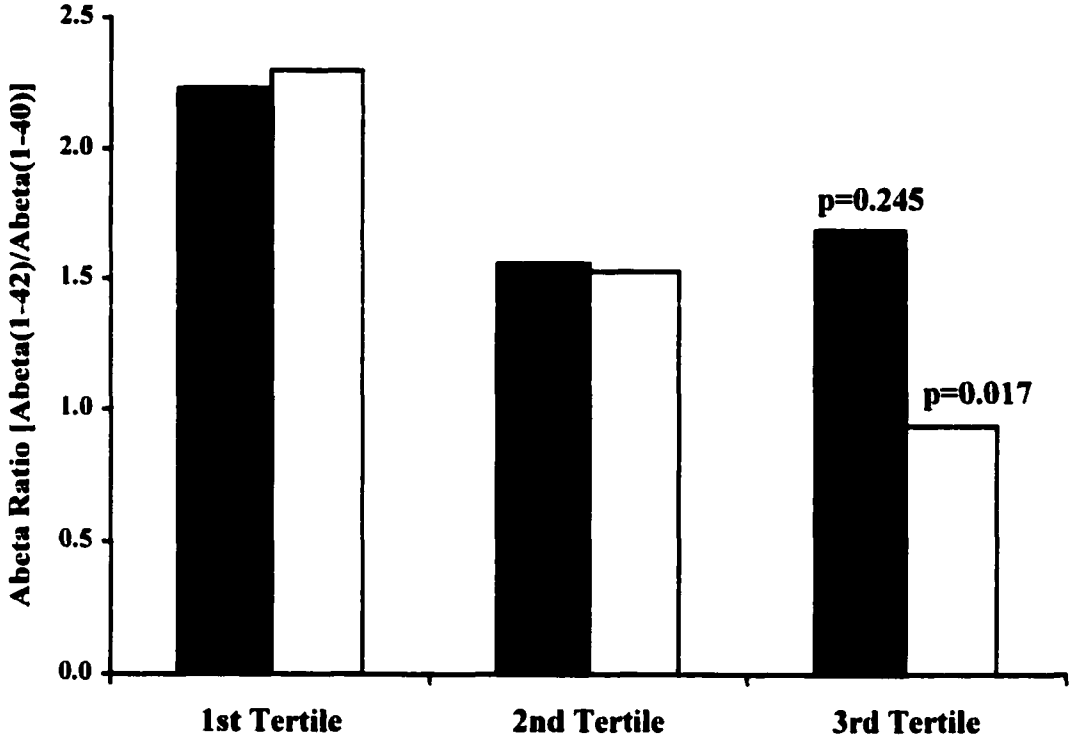

Figure 4.2 Abeta Ratio [Abeta(1-42)/Abeta(1-40)] Least-squares Means for workers with no (black bars) and some (white bars) physical work on the day of the blood draw by tertile of Standard Deviation of the mean magnetic field exposure, n = 60.[a]

[a] Means are adjusted for age, income, physical work, and vegetable consumption. p-values are for comparison of difference between means of 3rd and 1st tertiles.

Table 4.6. Comparison of least-squares means of Abeta Ratio by 1st and 3rd tertile of creatinine-adjusted post-workshift 6-OHMS at different exclusion cutpoints for difference in time between blood draw and end of workshift.[a]

Time Difference	N	1st Tertile	3rd Tertile	Difference	Percent change	p-value
All	60	1.56	1.16	-0.40	-26%	0.199
≤ 90 min.	46	1.39	0.88	-0.51	-37%	0.121
≤ 60 min.	37	1.79	0.98	-0.81	-45%	0.035
≤ 30 min.	23	2.02	0.84	-1.18	-58%	0.030

[a] Means are adjusted for rcm, age, income, physical work, and vegetable consumption.

CHAPTER 5

CONCLUSIONS

These studies were undertaken in order to assess the relationship between occupational magnetic field exposure and neurodegenerative disease. Relatively few epidemiological studies have been conducted on this association, and there have been no such investigations exploring the potential biological mechanisms. The findings presented here offer some support for our original hypotheses.

In the first study, it was hypothesized that individuals with Alzheimer's disease or ALS listed on their death certificate were more likely to have had occupations with magnetic field exposure than individuals without these diseases. When assessing exposure by electrical occupations or a multi-tiered categorization by job title and industry, we found elevated, statistically significant, risks for ALS. For Alzheimer's disease, there was a modestly elevated risk for only one of the three methods of exposure assessment. As discussed previously, there are several limitations in using death certificate data, including a potential differential bias in reporting of neurological disease with respect to occupation. For example, we observed elevated reporting of Alzheimer's disease among professional and managerial occupations despite the fact that previous studies have demonstrated these classes of occupations to be protective for the disease.[192-195] This indication of a diagnostic sensitivity bias could result in an underestimation of the true risk.

Neither Alzheimer's disease nor ALS demonstrated elevated risk when individuals were categorized by mean estimates of magnetic field exposure based on the JEM. There were several potential explanations for the discrepancy between results based on the first two methods of exposure assessment and the JEM. First, the positive findings observed when using electrical occupations or a combination of job title and industry could have been due to misclassification. As demonstrated when comparing these job titles with the JEM, some of these electrical occupations were not in the highest quartile of estimated exposure. Exposure misclassification would have been non-differential with respect to disease and would have biased results toward the null. The second potential explanation for the discrepancy between the exposure methods may be due to the limitations of the JEM. The JEM is based on measures of magnetic field intensity without indication of other magnetic field characteristics such as temporal coherence of the field or field orientation. It is possible that electrical occupations are associated with exposure to certain field characteristics in addition to field intensity that are important in terms of risk for these diseases. Recent human studies have demonstrated that temporal coherence and circularly or elliptically polarized fields are important in eliciting magnetic field-induced effects on the expression of 6-OHMS.[157,158,203] Finally, an exposure associated with electrical work other than magnetic fields may be responsible for the observed elevation in risk. For example, some epidemiological studies have demonstrated that antecedent electrical shock events are risk factors for ALS.[16,42,184] Individuals employed in electrical occupations would have greater opportunity for electric shock events compared to most other occupations.

Parkinson's disease and brain cancer were included in the study as a means of validating our methodology and were intended to be negative and positive comparison analyses, respectively. With the exception of a weak association between brain cancer and the highest quartile of JEM-estimated magnetic field exposure, we did not find strong support for a risk of brain cancer. This is difficult to explain given the fact that previous death certificate-based case-control studies consistently demonstrate a slight elevation in risk of brain cancer for individuals in electrical occupations. These studies have presented risk estimates as low as 1.4,[8] and we did not have adequate power to detect a true risk of that magnitude. Surprisingly, we found a modest increase in risk for Parkinson's disease among occupations considered to have high magnetic field exposure. It should be noted, however, that the use of Parkinson's disease as a negative comparison analysis was based on only two studies that specifically evaluated magnetic field exposure as a risk factor for this condition. The findings for these two diseases do not help in validating our methodology and may, in fact, weaken the conclusions that can be drawn from the Alzheimer's disease and ALS results. Further studies are needed to confirm the association between Parkinson's disease and occupational magnetic field exposure found in this study.

The second study focused on a protein present in the pathological lesions observed in Alzheimer's disease. It was hypothesized that concentrations of circulating Abeta are associated with occupational magnetic field exposure. No association between measures of magnetic field intensity and Abeta was found, but there was an inverse relationship between measures of magnetic field variability and Abeta. Individuals with high RCM values had relatively lower Abeta levels. This finding was not consistent with

previous epidemiological studies of Alzheimer's disease which were based on measures of magnetic field intensity.[9,10,13,14,17] Moreover, the results from four case-control studies were heavily dependent upon sewing machine operators,[9,10] and these magnetic field sources were shown to be highly variable.[223] By contrast, we found magnetic field variability to be protective for the biological marker, Abeta.

One method of acquiring high RCM values is through proximity to a constantly varying field source. An alternative explanation is that variability in magnetic field exposure was indicative of some other type of activity such as physical movement in and out of field sources throughout the work period. We found that individuals who were physically active at work were more likely to have lower Abeta concentrations. High RCM values may serve as a surrogate for movement or physical activity, explaining some of the variation in Abeta that was not adequately explained by the physical work variable. This argument is supported by the attenuation in the relationship between magnetic field variability and Abeta observed among those who had not engaged in physical work.

We also observed an inverse association between contemporary measures of the urinary melatonin metabolite, 6-OHMS, and Abeta. This was consistent with our original hypothesis, but we were unable to demonstrate a relationship between magnetic field exposure and 6-OHMS in this study population. Several environmental factors other than magnetic fields affect melatonin levels, including day-night cycles, season, and the exogenous administration of melatonin supplements. The importance of these factors in perturbing Abeta levels should be evaluated in future studies.

There have been several studies assessing the effects of genetic risk factors for Alzheimer's disease on Abeta expression in blood. To our knowledge, no previous

studies of the effects of environmental or physiological factors on Abeta expression *in vivo* have been conducted. Our findings suggest some avenues for future research. First, a follow-up study is required to confirm our findings of an association between magnetic field variability and Abeta. More rigorous assessment of individuals' physical activity and movement would be required to separate out this factor from magnetic field exposure metrics. Second, better assessment of the relationship between melatonin and Abeta would require more precise sample collection, as well as serial sampling to determine whether or not the expression of Abeta has a circadian or seasonal rhythm similar to melatonin. Finally, the ability to quantify circulating soluble Abeta in humans allows for future biomarker investigations of other factors that have been putatively associated with Alzheimer's disease such as estrogen deficiency and certain metals.

Taken together, the two studies presented here offered limited support for an association between occuational magnetic field exposure and risk of Alzheimer's disease. We did not find an association between Alzheimer's deaths and a history of occupations known to have high exposures to magnetic fields, with the exception of a weak association observed when using one of the three exposure assessment methods. However, the lack of an apparent association in the case-control study was not inconsistent with our finding that exposure to highly variable magnetic fields may perturb levels of circulating Abeta in electric utility workers. The methods of exposure assessment for the case-control study were based on measures of magnetic field intensity without regard to other potentially relevant field characteristics. Whether using historical job histories or concurrent personal measurements, these two studies reinforce the

importance of assessing field characteristics other than intensity in future studies of Alzheimer's disease and magnetic field exposure.

REFERENCES

1. Evans DA. Descriptive epidemiology of Alzheimer's disease. In: Khachaturian ZS, Radebaugh TS, eds. Alzheimer's disease: causes, diagnosis, treatment, and care. New York, NY: CRC Press, 1996.

2. Juergens SM, Kurland LT, Okazaki H, Mulder DW. ALS in Rochester, Minnesota, 1925-1977. Neurology 1980;30:463-470.

3. Murros K, Fogelholm R. Amyotrophic lateral sclerosis in Middle-Finland: an epidemiological study. Acta Neurology Scandinavia 1983;67:41-47.

4. Kahana E, Alter M, Feldman S. Amyotrophic lateral sclerosis: a population study. Journal of Neurology 1976;212:205-213.

5. Lilienfeld PE, Perl PD. Projected neurodegenerative disease mortality in the United States 1990-2010. Neuroepidemiology 1993;12:219-228.

6. U.S. Bureau of the Census. Projections of the population of the United States, by age, sex, and race: 1988 to 2080. Current population reports. Population Estimates and Projections Series P-25., 1989.

7. Advisory Panel on Alzheimer's Disease. Report of the advisory panel on Alzheimer's disease, 1988-1989.. Washington, DC: Supt. of Docs., U.S. Govt. Print. Off., 1989.

8. Kheifets LI, Afifi AA, Buffler PA, Zhang ZW. Occupational electric and magnetic field exposure and brain cancer: a meta-analysis. Journal of Occupational and Environmental Medicine 1995;37:1327-1341.

9. Sobel E, Davanipour Z, Sulkava R, Erkinjuntti T, Wilkstrom J, Henderson V, Buckwalter G, Bowman JD, Lee P. Occupations with exposure to electromagnetic fields: a possible risk factor for Alzheimer's disease. American Journal of Epidemiology 1995;142:515-524.

10. Sobel E, Dunn M, Davanipour Z, Qian Z, Chui HC. Elevated risk of Alzheimer's disease among workers with likely exposure to electromagnetic field exposure. Neurology 1996;47:1477-1481.

11. Schulte PA, Burnett CA, Boeniger MF, Johnson J. Neurodegenerative diseases: occupational occurrence and potential risk factors. American Journal of Public Health 1996;86:1281-1288.

12. Schulte PA, Burnett CA. EMFs and Alzheimer's disease (letter). Neurology 1997;49:312.

13. Savitz DA, Loomis DP, Tse CJ. Electrical occupations and neurodegenerative disease: analysis of U.S. mortality data. Archives of Environmental Health 1998;53(1):71-74.

14. Savitz DA, Checkoway H, Loomis DP. Magnetic field exposure and neurodegenerative disease mortality among electric utility workers. Epidemiology 1998;9(4):398-404.

15. Davanipour Z, Sobel E, Bowman J. Amyotrophic lateral sclerosis and occupational exposure to EMFs. Bioelectromagnetics 1997;18:28-35.

16. Deapen DM, Henderson BE. A case-control study of amyotrophic lateral sclerosis. American Journal of Epidemiology 1986;123(5):790-799.

17. Feychting M, Pedersen NL, Svedberg P, Floderus B, Gatz M. Dementia and occupational exposure to magnetic fields. Scandinavian Journal of Work, Environment and Health 1998;24(1):46-53.

18. Johansen C, Olsen C. Mortality from amyotrophic lateral sclerosis, other chronic disorders, and electric shocks among utlity workers. American Journal of Epidemiology 1998;148(4):362-368.

19. Katzman R. Current research on Alzheimer's disease in historical perspective. In: Khachaturian ZS, Radebaugh TS, eds. Alzheimer's disease: cause(s), diagnosis, treatment, and care. Boca Raton, FL: CRC Press, 1996.

20. Morris JC. Diagnosis of Alzheimer's disease. In: Khachaturian ZS, Radebaugh TS, eds. Alzheimer's disease: cause(s), diagnosis, treatment, and care. Boca Raton, FL: CRC Press, 1996.

21. Hardy J. Framing β-amyloid. Nature Genetics 1992;1:233-234.

22. Mayeux R. Putative risk factors for Alzheimer's disease. In: Khachaturian ZS, Radebaugh TS, eds. Alzheimer's disease: causes, diagnosis, treatment, and care. New York, NY: CRC Press, 1996.

23. Roses AD. The metabolism of apolipoprotein E and the Alzheimer's diseases. In: Khachaturian ZS, Radebaugh TS, eds. Alzheimer's disease: causes, diagnosis, treatment, and care. New York, NY: CRC Press, 1996.

24. Gatz M, Pedersen NL, Berg S, Johansson B, Johansson D, Mortimer JA, Posner SF, Viitanen M, Winblad B, Ahlbom A. Heritability of Alzheimer's disease: the study of dementia in Swedish twins. Journal of Gerontology 1997;52A(2):M117-M125.

25. Breitner JC, Welsh KA, Gau BA, McDonald WM, Steffans DC, Saunders AM, Magruder KM, Plassman BL, Folstein MF. Alzheimer's disease in the National Academy of Sciences - National Research Council Registry of Aging Twin Veterans. III. Detection of cases, longitudinal results, and observations on twin concordance. Archives of Neurology 1995;52(8):763-771.

26. Bergem ALM. Heredity of dementia of the Alzheimer type. Clinical Genetics 1994;46:144-149.

27. Raiha I, Kaprio J, Koshenvuo M, Rajala T, Sourander L. Alzheimer's disease in Finnish twins. Lancet 1996;347(9001):573-578.

28. Plassman BL, Breitner JCS. Recent advances in the genetics of Alzheimer's disease and vascular dementia with an emphasis on gene-environment interactions. Journal of the American Geriatric Society 1996;44:1242-1250.

29. Jorm AF. The epidemiology of Alzheimer's disease and related disorders. New York, NY: Chapman and Hall, 1990.

30. Roman GC, Tatemichi TK, Erkinjuntti T. Vascular dementia: diagnostic criteria for research studies. Neurology 1993;43:250-260.

31. Mirra SS, Markesbery WR. The neuropathology of Alzheimer's disease: diagnostic features and standardization. In: Khachaturian ZS, Radebaugh TS, eds. Alzheimer's disease: causes, diagnosis, treatment, and care. New York, NY: CRC Press, 1996.

32. Chou SM. Pathology of motor system disorder. In: Leigh PN, Swash M, eds. Motor neuron disease: biology and management. London: Springer-Verlag, 1995.

33. Swash M, Schwartz MS. Motor neuron disease: the clinical syndrome. In: Leigh PN, Swash M, eds. Motor neuron diseases: biology and management. London: Springer-Verlag, 1995.

34. Kondo K. Epidemiology of motor neuron disease. In: Leigh PN, Swash M, eds. Motor neuron disease: biology and management. London: Springer-Verlag, 1995.

35. De Belleroche J, Leigh PN, Rose FC. Familial motor neuron disease. In: Leigh PN, Swash M, eds. Motor neuron disease: biology and management. London: Springer-Verlag, 1995.

36. Estrin WJ. Amyotrophic lateral sclerosis in dizygotic twins. Neurology 1977;27(7):692-694.

37. Jokelainen M, Palo J, Lokki J. Monozygous twins discordant for amyotrophic lateral sclerosis. European Neurology 1978;17(5):296-299.

38. Strong MJ, Garruta RM. Motor neuron disease. In: Yasui M, Strong MJ, Ota K, A VM, eds. Mineral and metal neurotoxicology. New York, NY: CRC Press, 1997.

39. Nunn PB. Toxicology of motor neuron disease. In: Leigh PN, Swash M. eds. Motor neuron disease: biology and management. London: Springer-Verlag, 1995.

40. Kondo K, Tsubaki T. Case-control studies of motor neuron disease. Archives of Neurology 1981;38:220-226.

41. Kurtzke JF, Beebe GW. Epidemiology of amyotrophic lateral sclerosis: a case-control comparison based on ALS deaths. Neurology 1980;30:453-462.

42. Gawel M, Zaiwalla Z, Rose FC. Antecedent events in motor neuron disease. Journal of Neurology, Neurosurgery, and Psychiatry 1983;46:1041-1043.

43. Savettieri G, Salemi G, Arcara A, Cassata M, Castiglione MG, Fierro B. A case-control study of amyotrophic lateral sclerosis. Neuroepidemiology 1991;10:242-245.

44. Gunnarsson L, Bodin L, Soderfeldt B, Axelson O. A case-control study of motor neurone disease: its relation to heritability, occupational exposures, particularly to solvents. British Journal of Industrial Medicine 1992;49:791-798.

45. Cruz DC, Nelson LM, McGuire V, Longstreth WT. Physical trauma and family history of neurodegenerative diseases in amyotrophic lateral sclerosis: a population-based case-control study. Neuroepidemiology 1999;18(2):101-110.

46. Gunnarsson L, Lindberg G, Soderfeldt B, Axelson O. Amyotrophic lateral sclerosis in Sweden in relation to occupation. Acta Neurology Scandinavia 1991;83:394-398.

47. Strickland D, Smith SA, Dolliff G, Goldman L, Roelofs RI. Amyotrohpic lateral sclerosis and occupational history: a pilot case-control study. Archives of Neurology 1996;53:730-733.

48. Nicholas JS, Lackland DT, Dosemeci M, Mohr LC, Dunbar JB, Grosche B, Hoel DG. Mortality among US commercial pilots and navigators. Journal of Occupational and Environmental Medicine 1998;40(11):980-985.

49. Davanipour Z, Sobel E, Vu H, Will AD. Electromagnetic field exposure and amyotrophic lateral sclerosis (letter). Neuroepidemiology 1991;10:308.

50. Rajput AH. Current concepts in the etiology of Parkinson's disease. In: Schneider JS, Gupta M, eds. Current concepts in Parkinson's disease research. Seattle, WA: Hogrefe and Huber Publishers, 1993.

51. Brody JA, Hirano A, Scott RM. Recent neuropathologic observations in amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. Neurology 1971;21:528-537.

52. Langston JW, Ballard P. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983;219:979-980.

53. Langston JW. Epidemiology versus genetics in Parkinson's disease: progress in resolving an age-old debate. Annals of Neurology 1998;44(Suppl 1):S45-S52.

54. Milham S. Mortality in workers exposed to electromagnetic fields. Environmental Health Perspectives 1985;62:297-300.

55. Reif JS, Pearce N, Fraser J. Occupational risks for brain cancer: a New Zealand cancer registry-based study. Journal of Occupational Medicine 1989;31(10):863-867.

56. Magnani C, Coggon D, Osmond C, Acheson ED. Occupation, five cancer: a case-control study using death certificates. British Journal of Industrial Medicine 1987;44:769-776.

57. Floderus B, Persson T, Stenlund C, Wennberg A, Ost A, Knave B. Occupational exposure to electromagnetic fields in relation to leukemia and brain tumors: a case-control study in Sweden. Cancer Causes and Control 1993;4:465-476.

58. Demers PA, Vaughan RL, Schommer RR. Occupation, socio-economic status, and brain tumor mortality: a death certificate-based case-control study. Journal of Occupational Medicine 1991;339(1001-1005).

59. Ryan P, Lee MW, North JB, McMichael J. Risk factors for tumors of the brain and meninges: results from the Adelaide adult brain tumor study. International Journal of Cancer 1992;51:20-27.

60. Lin RS, Dischinger PC, Conde J, Farrell KP. Occupational exposure to electromagnetic fields and the occurrence of brain tumors: an analysis of possible associations. Journal of Occupational Medicine 1985;276:413-419.

61. Speers MA, Dobbins JG, Miller VS. Occupational exposures and brain cancer mortality: a preliminary study of East Texas residents. American Journal of Industrial Medicine 1988;13:629-638.

62. Thomas TL, Stolley PD, Stemhagen A, Fontham ETH, Bleecker ML, Stewart PA, Hoover RN. Brain tumor mortality risk among men with electrical and electronics jobs: a case-control study. Journal of the National Cancer Institute 1987;79:233-238.

63. Theriault G, Goldberg M, Miller AB, Armstrong B, Guenel P, Deadman J, Imbernon E, To T, Chevalier A, Cyr D, Wall C. Cancer risks associated with occupational exposure to magnetic fields among electric utility workers in Ontario and Quebec, Canada, and France: 1970-1989. American Journal of Epidemiology 1994;139(6):550-572.

64. Loomis DP, Savitz DA. Mortality from brain cancer and leukaemia among electrical workers. British Journal of Industrial Medicine 1990;47:633-638.

65. Preston-Martin S, Staples M, Farrugia H, Giles G. Primary tumors of the brain, cranial nerves and cranial meninges in Victoria, Australia, 1982-1990: patterns of incidence and survival. Neuroepidemiology 1993;12:270-279.

66. Schlehofer B, Kunze S, Sachsenheimer W, Blettner M, Niehoff D, Wahrendorf J. Occupational risk factors for brain tumors: results from a population-based case-control study in Germany. Cancer Causes and Control 1990;1:209-215.

67. Feychting M, Forssen U, Floderus B. Occupational and residential magnetic field exposure and leukemia and central nervous system tumors. Epidemiology 1997;8(4):384-389.

68. Harrington JM, McBride DI, Sorahan T, Paddle GM, van Tongeren M. Occupational exposure to magnetic fields in relation to mortality from brain cancer among electricity generation and transmission workers. Occupational and Environmental Medicine 1997;54:7-13.

69. Johansen C, Olsen JH. Risk of cancer among Danish utility workers - a nationwide cohort study. American Journal of Epidemiology 1998;147(6):548-555.

70. Fear NT, Roman E, Carpenter LM, Newton R, Bull D. Cancer in electrical workers: an analysis of cancer registrations in England, 1981-87. British Journal of Cancer 1996;73:935-939.

71. Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochemical and Biophysical Research Communications 1984;120:885-890.

72. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proceedings of the National Academy of Science USA 1985;82:4245-4249.

73. Selkoe DJ, Bell DS, Podlisny MB, Price DL, C CL. Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. Science 1987;235:873-877.

74. Probst A, Langui D, Ipsen S, Robakis N, Ulrich J. Deposition of β/A4 protein along neuronal plasma membranes in diffuse senile plaques. Acta Neuropathology 1991;83:21-29.

75. Roher A, Wolfe D, Palutke M, KuKurga D. Purification, ultrastructure, and chemical analysis of Alzheimer disease amyloid plaque core protein. Proceedings of the National Academy of Science USA 1986;833:2662-2666.

76. Bush AI, Martins RN, Rumble B, Moir R, Fuller S, Milwar E, Currie J, Ames D, Weidemann A, Fischer P, Multhaup G, Beyreuther K, L MC. The amyloid precursor protein of Alzheimer's disease is released by human platelets. Journal of Biological Chemistry 1990;265:15977-15983.

77. Monning U, Konig G, Prior R, Mechler H, Schreiter-Gasser U, Master CL, Beyreuther K. Synthesis and secretion of Alzheimer amyloid βA4 precursor protein by stimulated human peripheral blood leucocytes. FEBS Letters 1990;277:261-266.

78. Schubert D, Jin L-W, Saitoh T, Cole G. The regulation of amyloid β protein precursor secretion and its modulatory role in cell adhesion. Neuron 1989;3:689-694.

79. Saitoh T, Sundsmo M, Roch J-M, Kimura T, Cole G, Schubert D, Oltersdorf T, Schenk DB. Secreted form of amyloid β protein precursor is involved in the growth regulation of fibroblasts. Cell 1989;58:615-622.

80. Checler F. Processing of the β-amyloid precursor protein and its regulation in Alzheimer's disease. Journal of Neurochemistry 1995;65(4):1431-1444.

81. Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo IH, Schenk D, Teplow DB, Selkoe DJ. Amyloid β-peptide is produced by cultured cells during normal metabolism. Nature 1992;359:322-325.

82. Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, Cai X, McKay DM, Tintner R, Frangione B, Younkin SG. Production of the Alzheimer amyloid β protein by normal proteolytic processing. Science 1992;258:126-129.

83. Selkoe DJ. Physiological production of β-amyloid protein and the mechanism of Alzheimer's disease. Trends in Neurological Science 1993;16:403-409.

84. Wisniewski KE, Wisniewski HM, Wen GY. Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. Annals of Neurology 1985;17:278-282.

85. Teller JK, Russo C, DeBusk LM, Angelini G, Zaccheo D, Dagna-Bricarelli F, Scartezzini P, Bertolini S, Mann DMA, Tabaton M, Gambetti P. Presence of soluble amyloid β-peptide precedes amyloid plaque formation in Down's syndrome. Nature Medicine 1996;2:93-95.

86. Suzuki N, Cheung TT, Cai X, Odaka A, Otvos L, Eckman C, Golde TE, Younkin SG. An increased percentage of long amyloid β protein secreted by familial amyloid β protein precursor (βAPP717) mutants. Science 1994;264:1336-1340.

87. Citron M, Vigo-Pelfrey C, Teplow DB, Miller C, Schenk D, Johnston J, Winblad B, Venizelos N, Lannfelt L, Selkoe DJ. Excessive production of amyloid β-protein by peripheral cells of symptomatic and presymptomatic patients carrying the Swedish familial Alzheimer's disease mutation. Proceedings of the National Academy of Science USA 1994;91:11993-11997.

88. Citron M, Eckman CB, Diehl TS, Corcoran C, Ostaszewski BL, Xia W, Levesque G, St. George-Hyslop P, Younkin SG, Selkoe DJ. Additive effects of PS1 and APP mutations on secretion of the 42-residue amyloid β-protein. Neurobiology of Disease 1998;5:107-116.

89. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Vitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nature Medicine 1996;2(8):864-870.

90. Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada C, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS. Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1-42/1-40 ratio *in vitro* and *in vivo*. Neuron 1996;17:1005-1013.

91. Joachim CL, Mori H, Selkoe DJ. Amyloid β-protein deposition in tissues other than brain in Alzheimer's disease. Nature 1989;341:226-230.

92. Soininen H, Syrjanen S, Heinonen O, Neittaanmaki H, Meittinne R, Paljarvi L, Syrjanen K, Beyreuther K, Riekkinen P. Amyloid β-protein deposition in skin of patients with dementia. Lancet 1992;339:245.

93. Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C, McCormach R, Wolfert R, Selkoe D, Lieberburg I, Schenk D. Isolation and quantification of soluble Alzheimer's β-peptide from biological fluids. Nature 1992;359:325-327.

94. Tamaoka A, Fukushima T, Sawamura N, Ishikawa K, Oguni E, Komatsuzaki Y, Shoji S. Amyloid β protein in plasma from patients with sporadic Alzheimer's disease. Journal of Neurological Sciences 1996;151:65-68.

95. Tamaoka A, Sawamura N, Fukushima T, Shoji S, Matsubara E, Shoji M, Hirai S, Furiya Y, Endoh R, Mori H. Amyloid β protein 42(3) in cerbrospinal fluid of patients with Alzheimer's disease. Journal of Neurological Sciences 1997;148:41-45.

96. Podlinsky MB, Mammen AL, Scholssmacher MG, Palmert MR, Younkin SG, Selkoe DJ. Detection of soluble forms of the β-amyloid precursor protein in human plasma. Biochemical and Biophysical Research Communications 1990;167:1094-1101.

97. Ghiso J, Wisniewski T, Frangione B. Unifying features of systemic and cerebral amyloidosis. Molecular Neurobiology 1994;8:49-64.

98. Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A, Yates J, Cotman C, Glabe C. Assembly and aggregation properties of synthetic Alzheimer's A4/β amyloid peptide analogs. Journal of Biological Chemistry 1992;267:546-554.

99. Ghersi-Egea JF, Gorevic PD, Ghiso J, Frangione B, Patlak CS, Fenstermacher JD. Fate of cerbrospinal fluid-borne amyloid β-peptide: rapid clearance into blood and appreciable accumulation by cerebral arteries. Journal of Neurochemistry 1996;67:880-883.

100. Weller RO, Massey A, Newman TA, Hutchings M, Kuo Y-M, Roher AE. Cerebral amyloid angiopathy: amyloid β accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. American Journal of Pathology 1998;153(3):725-733.

101. Tokuda T, Fukushima T, Ikeda S, Sekijima Y, Shoji S, Yanagisawa N, Tamaoka A. Plasma levels of amyloid β proteins Aβ1-40 and Aβ1-42(3) are elevated in Down's Syndrome. Annals of Neurology 1997;41:271-273.

102. Mehta PD, Dalton AJ, Mehta SP, Kim KS, Sersen EA, Wisniewski HM. Increased plasma amyloid β protein 1-42 levels in Down's syndrome. Neuroscience Letters 1998;241:13-16.

103. Kosaka T, Imagawa M, Seki K, Arai H, Sasaki H, Tsuji S, Asami-Odaka A, Fukushima T, Imai K, Iwatsubo T. The βAPP717 Alzheimer mutation increases the percentage of plasma amyloid-β protein ending at Aβ42(3). Neurology 1997;48:741-745.

104. Iwatsubo T. Amyloid β protein in plasma as a diagnostic marker for Alzheimer's disease. Neurobiology of Aging 1998;19(2):161-163.

105. Zlokovic BV, Ghiso J, Mackic JB, McComb JG, Weiss MH, Frangione B. Blood-brain barrier transport of circulating Alzheimer's amyloid β. Biochemical and Biophysical Research Communications 1993;197:1034-1040.

106. Mannes LM, Banks WA, Podlinsky MB, Selkoe DJ, Kastin AJ. Passage of human amyloid β-protein 1-40 across the murine blood-brain barrier. Life Sciences 1994;55:1643-1650.

107. Martel CL, Mackic JB, McComb JG, Ghiso J, Zlokovic BV. Blood-brain barrier uptake of the 40 and 42 amino acid sequences of circulating Alzheimer's amyloid β in guinea pig brains. Neuroscience Letters 1996;206:157-160.

108. Banks WA, Kastin AJ, Maness LM, Banks MF, Shayo M, McLay RN. Interactions of β-amyloids with the blood-brain barrier. Annals of the New York Academy of Sciences 1997;826:190-199.

109. Zlokovic BV, Martel CL, Mackic JB, Matsubara E, Wisniewski T, McComb JG, Frangione B, Ghiso J. Brain uptake of circulating apolipoproteins J and E complexed to Alzheimer's amyloid β. Biochemical and Biophysical Research Communications 1994;205:1431-1437.

110. Martel CL, Mackic JB, Matsubara E, Governale S, Miguel C, Miao W, McComb JG, Frangione B, Ghiso J, Zlokovic BV. Isoform-specific effects of apolipoproteins E2, E3, and E4 on cerebral capillary sequestration and blood-brain barrier transport of circulating Alzheimer's amyloid β. Journal of Neurochemistry 1997;69:1995-2004.

111. Yang D-S, Smith JD, Zhuo Z, Gandy SE, Martins RN. Characterization of the binding of amyloid-β peptide to cell culture-derived native apolipoprotein E2, E3, and E4 isoforms and to isoforms from human plasma. Journal of Neurochemistry 1997;68:721-725.

112. Aleshkov S, Abraham CR, Zannis VI. Interaction of nascent apoE2, apoE3, and apoE4 isoforms expressed in mammalian cells with amyloid peptide β(1-40). Relevance to Alzheimer's disease. Biochemistry 1997;36(34):10571-10580.

113. Ellis RJ, Olichney JM, Thal LJ, Mirra SS, Morris JC, Beekly D, Heyman A. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, part XV. Neurology 1996;46:1592-1596.

114. Kalaria RK, Hedera P. Differential degeneration of the cerebral microvasculature in Alzheimer's disease. NeuroReport 1995;6(3):477-480.

115. Premkumar DRD, Cohen DL, Hedera P, Friedland RP, Kalaria RN. Apolipoprotein E-ε4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease. American Journal of Pathology 1996;148(6):2083-2095.

116. Wisniewski HM, Vorbrodt AW, Wegiel J. Amyloid angiopathy and blood-brain barrier changes in Alzheimer's disease. Annals of the New York Academy of Sciences 1997;826:161-172.

117. Crawford F, Suo Z, Fang C, Sawar A, Su G, Arendash G, Mullan M. The vasoactivity of Aβ peptides. Annals of the New York Academy of Sciences 1997;826:35-46.

118. Thomas T, Thomas G, McLendon C, Sutton T, Mullan M. β-amyloid-mediated vasoactivity and vascular endothelial damage. Nature 1996;380:168-171.

119. Thomas T, McLendon C, Sutton ET, Thomas G. Cerebrovascular endothelial dysfunction mediated by β-amyloid. NeuroReport 1997;8(6):1387-1391.

120. Suo Z, Humphrey J, Kundtz A, Sethi F, Placzek A, Crawford F, Mullan M. Soluble Alzheimers β-amyloid constricts the cerebral vasculature in vivo. Neuroscience Letters 1998;257:77-80.

121. Paris D, Town T, Parker TA, Humphrey J, Mullan M. Isoform-specific vasoconstriction induced by apolipoprotein E and modulation of this effect by Alzheimer's β-amyloid peptide. Neuroscience Letters 1998;256:73-76.

122. Gibson GE, Peterson C. Calcium and the aging nervous system. Neurobiology of Aging 1987;8:329-343.

123. Penner R, Neher E. The role of calcium in stimulus-secretion coupling in excitable and non-excitable cells. Journal of Experimental Biology 1988;139:329-345.

124. Baker PF, Knight DE. Exocytosis: control by calcium and other factors. British Medical Bulletin 1986;42(4):399-404.

125. Matthews EK. Calcium and membrane permeability. British Medican Journal 1986;42:391-397.

126. Bawin SM, Adey WR. Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. Proceedings of the National Academy of Science USA 1976;73:1999-2003.

127. Blackman CF. ELF effects on calcium homeostasis. In: Wilson BW, Stevens RG, Anderson LE, eds. Extremely low frequency magnetic fields: the question of cancer. Columbus, OH: Battelle Press, 1990.

128. Lindstrom E, Lindstrom P, Berglund A, Hansson-Mild K, Lundgren E. Intracellular calcium oscillations induced in a T-cell line by a weak 50 Hz magnetic field. Journal of Cellular Physiology 1993;156:395-398.

129. Lindstrom E, Lindstrom P, Berglund A, Lundgren E, Hansson-Mild K. Intracellular calcium oscillations in a T-cell line after exposure to extremely-low-frequency magnetic fields with variable frequencies and flux densities. Bioelectromagnetics 1995;16:41-47.

130. Yost MG, Liburdy RP. Time-varying and static magnetic fields acts in combination to alter calcium signal transduction in the lymphocyte. FEBS Letters 1992;296:117-122.

131. Walleczek J, Liburdy RP. Nonthermal 60 Hz sinusoidal magnetic-field exposure enhances $^{45}Ca^{2+}$ uptake in rat thymocytes: dependence on mitogen activation. FEBS Letters 1990;271(1,2):157-160.

132. Liboff AR. Electric-field ion cyclotron resonance. Bioelectromagnetics 1997;18:85-87.

133. Siesjo BK. Calcium-mediated processes in neuronal degeneration. Annals of the New York Academy of Sciences 1994;747:140-161.

134. Buxbaum JD, Ruefli AA, Parker CA, Cypess AM, Greengard P. Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. Proceedings of the National Academy of Science USA 1994;91:4489-4493.

135. Querforth HW, Selkoe DJ. Calcium ionophore increases amyloid β peptide production by cultured cells. Biochemistry 1994;33:4550-4561.

136. Hiraoka M, Miyakoshi J, Li YP, Shung B, Takebe H, Abe M. Induction of *c-fos* gene expression by exposure to a static magnetic field in HeLaS3 cells. Cancer Research 1992;52:6522-6524.

137. Lagroye I, Poncy JL. Influence of 50-Hz magnetic fields and ionizing radiation on c-jun and c-fos oncoproteins. Bioelectromagnetics 1998;19:112-116.

138. Rao S, Henderson AS. Regulation of c-fos is affected by electromagnetic fields. Journal of Cellular Biochemistry 1996;63:358-365.

139. Phillips JL, Haggren W, Thomas WJ, Ishida-Jones T, Adey WR. Magnetic field-induced changes in specific gene transcription. Biochimica et Biophysica Acta 1992;1132:140-144.

140. Parker JE, Winters W. Expression of gene-specific RNA in cultured cells exposed to rotating 60-Hz magnetic fields. Biochemistry and Cell Biology 1992;70:237-241.

141. Owen RD. MYC mRNA abundance is unchanged in subcultures of HL60 cells exposed to power-line frequency magnetic fields. Radiation Research 1998;150(1):23-30.

142. Lin H, Han L, Blank M, Head M, Goodman R. Magnetic field activation of protein-DNA binding. Journal of Cellular Biochemistry 1998;70:297-303.

143. Trejo J, Massamiri T, Deng T, Dewji NN, Bayney RM, Brown JH. A direct role for protein kinase C and the transcription factor jun/AP-1 in the regulation of Alzheimer's disease. Journal of Biological Chemistry 1994;269:21682-21690.

144. Lin H, Head M, Blank M, Han L, Jin M, Goodman R. *Myc*-mediated transactivation of HSP70 expression following exposure to magnetic fields. Journal of Cellular Biochemistry 1998;69:181-188.

145. Blank M, Khordova O, Goodman R. Changes in polypeptide distribution stimulated by different levels of electromagnetic and thermal stress. Bioelectrochemistry and Bioenergetics 1994;33:109-114.

146. Goodman R, Blank M, Lin H, Dai R, Khorkova O, Soo L, Weisbrot D, Henderson A. Increased levels of hsp70 transcripts induced when cells are exposed to low frequency electromagnetic fields. Bioelectrochemistry and Bioenergetics 1994;33:115-120.

147. Weisbrot DR, Khorkova O, Lin H, Henderson AR, Goodman R. The effect of low frequency electric and magnetic fields on gene expression in saccharomycescerevisiae. Bioelectrochemistry and Bioenergetics 1993;31:167-177.

148. Johnson G, Refolo LM, Wallace W. Heat-shocked neuronal PC12 cells reveal Alzheimer's disease-associated alterations in amyloid precursor protein and tau. Annals of the New York Academy of Sciences 1994;747:194-197.

149. Tomimoto H, Wakita H, Akiguchi I, Nakamura S, Kimura J. Temporal profiles of accumulation of amyloid β/A4 protein precursor in the gerbil after graded ischemic stress. Journal of Cerebral Blood Flow and Metabolism 1994;14:565-573.

150. Pappolla MA, Sambamurti K, Efthimiopoulus S, Refolo L, Omar RA, Robakis NK. Heat-shock induces abnormalities in the cellular distribution of amyloid precursor protein (APP) and APP fusion proteins. Neuroscience Letters 1995;192:105-108.

151. Brzezinski A. Melatonin in humans. New England Journal of Medicine 1997;336(3):186-194.

152. Stevens RG, Davis S. The melatonin hypothesis: electric power and breast cancer. Environmental Health Perspectives 1996;104(Suppl 1):135-140.

153. Graham C, Cook MR, Riffle DW, Gerkovich MM, Cohen HD. Nocturnal melatonin levels in human volunteers exposed to intermittant 60 Hz magnetic fields. Bioelectromagnetics 1996;17(4):263-273.

154. Graham C, Cook MR, Riffle DW. Human melatonin during continuous magnetic field exposure. Bioelectromagnetics 1997;18(2):166-171.

155. Selmaoui B, Lambrozo J, Touitou Y. Magnetic fields and pineal function in humans: evaluation of nocturnal acute exposure to extremely low frequency magnetic fields on serum melatonin and urinary 6-sulphatoxymelatonin circadian rhythms. Life Science 1996;58(18):1539-1549.

156. Wood AW, Armstrong SM, Sait ML, Devine L, J MM. Changes in human plasma melatonin profile in response to 50 Hz magnetic field exposure. Journal of Pineal Research 1998;25:116-127.

157. Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Reduced excretion of a melatonin metabolite in workers exposed to 60 Hz magnetic fields. American Journal of Epidemiology 1999;150(1):27-36.

158. Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Nocturnal exretion of a urinary melatonin metabolite in electric utility workers. Scandinavian Journal of Work, Environment and Health 1998;24:183-189.

159. Pfluger DH, Minder CE. Effects of exposure to 16.7 Hz magnetic fields on urinary 6-hydroxymelatonin sulfate excretion of Swiss railway workers. Journal of Pineal Research 1996;21:91-100.

160. Reiter RJ. The pineal gland and melatonin in relation to aging: a summary of the theories and of the data. Experimental Gerontology 1995;30(3/4):199-212.

161. Skene DJ, Vivien-Roels B, Sparks DL, Hunsaker JC, Pevet P, Ravid D, Swaab DF. Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human pineal gland: effect of age and Alzheimer's disease. Brain Research 1990;528:170-174.

162. Dori D, Casale G, Solerte SB, Fioravanti M, Migliorati G, Cuzzoni G, Ferrari E. Chrono-neuroendrocrinological aspects of physiological aging and senile dementia. Chronobiologia 1994;21:121-126.

163. Nair NPV, Hariharasubramanian N, Pilapil C, Thavunday JX. Plasma melatonin rhythm in normal aging and Alzheimer's disease. Journal of Neural Transmission 1986;21(Suppl):494.

164. Tohgi H, Abe T, Takahashi S, Kimura M, Takahashi J, Kikuchi T. Concentrations of serotonin and its related substances in the cerebrospinal fluid of patients with Alzheimer type dementia. Neuroscience Letters 1992;141:9-12.

165. Song W, Lahiri DK. Melatonin alters the metabolism of the β-amyloid precursor protein in the neuroendocrine cell line PC12. Journal of Molecular Neuroscience 1997;9:75-92.

166. Lahiri DK. Melatonin affects the metabolism of the beta-amyloid precursor protein in different cell types. Journal of Pineal Research 1999;26(3):137-146.

167. Arispe N, Pollard HB, Rojas E. The ability of amyloid β-protein [AβP(1-40)] to form Ca^{2+} channels provides a mechanism for neuronal death in Alzheimer's disease. Annals of the New York Academy of Sciences 1994;747:256-266.

168. Barger SW, Smith-Swintosky VL, Rydel RE, Mattson MP. β-amyloid precursor protein mismetabolism and loss of calcium homeostasis in Alzheimer's disease. Annals of the New York Academy of Sciences 1994;747:158-163.

169. Zhou Y, Gopalakrishnan V, Richardson JS. Actions of neurotoxic β-amyloid on calcium homeostasis and viability of PC12 cells are blocked by antioxidants but not by calcium channel antagonists. Journal of Neurochemistry 1996;67:1419-1425.

170. Pappolla M. Sos M. Omar RA, Bick RJ, Hickson-Bick DLM, Reiter RJ, Efthimiopoulos S, Robakis NK. Melatonin prevents death of neuroblastoma cells exposed to the Alzheimer amyloid peptide. Journal of Neuroscience 1997;17(5):1683-1690.

171. Chen LD, Tan DX, Reiter RJ, Yang K, Poeggler B, Kumar P, Manchester LC, Chambers JP. In vivo and in vitro effects on the pineal gland and melatonin on [Ca2+ + Mg2+]-dependent ATPase in cardiac sarcolemma. Journal of Pineal Research 1993;14(4):178-183.

172. Tan D-X, Chen L-D, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocrine Journal 1993;1:57-60.

173. Tan D-X, Poeggeler B, Reiter RJ, Chen L-D, Chen S, Manchester LC, Barlow-Walden LR. The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in vivo. Cancer Letters 1993;70:65-71.

174. Barlow-Walden LR, Reiter RJ, Abe M, Pablos M, Menedez-Pelaez A, Chen L-D, Poeggeler B. Melatonin stimulates brain glutathione peroxidase activity. Neurochemistry International 1995;26(5):497-502.

175. Pablos MI, Agapito MT, Gutierrez R, Recio JM, Reiter RJ, Barlow-Walden LR, Acuna-Castroviejo D, Menendez-Pelaez A. Melatonin stimulates the activity of glutathione peroxidase in several tissues of chicks. Journal of Pineal Research 1995;19:111-115.

176. Kotler M, Rodriguez C, Sainz RM, Antolin I, Menendez-Pelaez A. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. Journal of Pineal Research 1998;24:83-89.

177. Manev H, Tolga U, Kharlamov A, Joo J-Y. Increased brain damage after stroke or excitotoxic seizures in melatonin-deficient rats. FASEB Journal 1996;10:1546-1551.

178. Frederikse PH, Garland D, Zigler JS, Piatigorsky J. Oxidative stress increases production of β-amyloid precursor protein and β-amyloid (Aβ) in mammalian lenses, and Aβ has toxic effects on lens epithelial cells. Journal of Biological Chemistry 1996;271:10169-10174.

179. Yan SD, Yan SF, Chen X, Fu J, Chen M, Kuppusamy P, Smith MA, Perry G, Goodman GC, Nawroth P, Zweier JL, Stern D. Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid β-peptide. Nature Medicine 1995;1:693-699.

180. Zhang L, Zhao B, Yew DT, Kusiak JW, Roth GS. Processing of Alzheimer's amyloid precursor protein during H2O2-induced apoptosis in human neuronal cells. Biochemical and Biophysical Research Communications 1997;235:845-848.

181. Pappolla MA, Sos M, Omar RA, Sambamurti K. The heat shock/oxidative stress connection: relevance to Alzheimer's disease. Molecular and Chemical Neuropathology 1996;28:21-34.

182. U. S. Bureau of the Census. 1980 census of population: classified index of industries and occupations, 1980.

183. Touchstone JA, Bowman JD, Yost MG. A magnetic field job exposure matrix for Bureau of the Census 1980 codes. . Bioelectromagnetics Twenty-first Annual Meeting. Long Beach, CA, 1999;p233.

184. Gallagher JP, Sanders M. Trauma and amyotrophic lateral sclerosis: a report of 78 patients. Acta Neurology Scandinavia 1987;75(2):145-150.

185. Wechsler LS, Checkoway H, Franklin GM, Costa LG. A pilot study of occupational and environmental risk factors for Parkinson's disease. Neurotoxicology 1991;12:387-392.

186. Hoel DG, Ron E, Carter R, Mabuchi K. Influence of death certificate errors on cancer mortality trends. Journal of the National Cancer Institute 1993;85:1063-1068.

187. Percy C, Stanek E, Gloeckler L. Accuracy of cancer death certificates and its effect on cancer mortality statistics. American Journal of Public Health 1981;71(3):242-250.

188. Betemps EJ, Buncher CR. Birthplace as a risk factor in motor neuron disease and Parkinson's disease. International Journal of Epidemiology 1993;22:898-904.

189. Olichney JM, Hofstetter CR, Galasko D, Thal LJ, Katzman R. Death certificate reporting of dementia and mortality in an Alzheimer's disease research center cohort. Journal of the American Geriatric Society 1995;43:890-893.

190. Raiford K, Anton-Johnson S, Haycox Z, Nolan K, Schaffer A, Caimano C, Fillenbaum G, Heyman A. CERAD part VII: accuracy of reporting dementia on death certificates of patients with Alzheimer's disease. Neurology 1994;44:2208-2209.

191. Thomas T, Waxweiler R. Brain tumors and occupational risk factors: a review. Scandinavian Journal of Work, Environment and Health 1986;12:1-15.

192. Stern Y, Gurland B, Tatemichi TK, Tang MX, Wilder D, Mayeux R. Influence of education and occupation on the incidence of Alzheimer's disease. JAMA 1994;271:1004-1010.

193. The Candian Study of Health and Aging. The Canadian study of health and aging: risk factors for Alzheimer's disease in Canada. Neurology 1994;44:2073-2080.

194. Launer LJ, Andersen K, Dewey ME, Letenneur L, Ott A, Amaducci LA, Brayne C, Copeland JRM, Dartigues JF, Kragh-Sorensen P, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A. Rates and risk factors for dementia and Alzheimer's disease: results from EURODEM pooled analysis. Neurology 1999;52:78-84.

195. Zhang MY, Katzman R, Salmon D, Jin H, Cai GJ, Wang ZY, Qu GY, Grant I, Yu E, Levy P. The prevalence of dementia and Alzheimer's disease in Shanghai, China: impact of age, gender, and education. Annals of Neurology 1990;27(4):428-437.

196. Swanson GM, Schwartz AG, Burrows RW. An assessment of occupation and industry data from death certificates and hospital medical records for population-based cancer surveillance. American Journal of Public Health 1984;74(5):464-467.

197. Steenland K, Beaumont J. The accuracy of occupational and industry data on death certificates. Journal of Occupational Medicine 1984;26(4):288-296.

198. Schumacher MC. Comparison of occupation and industry information from death certificates and interviews. American Journal of Public Health 1986;76(6):635-637.

199. Turner DW, Schumacher MC, West DW. Comparison of occupational interview data to death certificate data in Utah. American Journal of Industrial Medicine 1987;12:145-151.

200. Andrews KW, Savitz DA. Accuracy of industry and occupation on death certificates of electric utility workers: implications for epidemiologic studies of magnetic fields and cancer. Bioelectromagnetics 1999;20:512-518.

201. Karasek M, Woldanska-Okonska M, Czernicki J, Zylinska K, Swietoslawski J. Chronic exposure to 2.9 mT, 40 Hz magnetic field reduces melatonin concentrations in humans. Journal of Pineal Research 1998;25:240-244.

202. Smith CCT. Stimulated release of the β-amyloid protein of Alzheimer's disease by normal human platelets. Neuroscience Letters 1997;235:157-159.

203. Burch JB, Reif JS, Noonan CW, Yost MG. Melatonin metabolite levels in workers exposed to 60 Hz magnetic fields: work in substations and with 3-phase conductors. Journal of Occupational and Environmental Medicine 1999;(In press).

204. Yost M. Alternative magnetic field exposure metrics: occupational measurements in trolley workers. Radiation Protection Dosimetry 1999;83(1-2):99-106.

205. Bracken TD, Patterson RM. Variability and consistency of electric and magnetic field occupational exposure measurements. Journal of Exposure Analysis and Environmental Epidemiology 1996;6(3):355-374.

206. Farrell JM, Barber M, Krause D, Litovitz TA. The superposition of a temporally incoherent magnetic field inhibits 60 Hz-induced changes in the ODC activity of developing chick embryos. Bioelectromagnetics 1998;19(1):53-56.

207. Litovitz TA, Krause D, Montrose CJ, Mullins JM. Temporally incoherent magnetic fields mitigate the response of biological systems to temporally coherent magnetic fields. Bioelectromagnetics 1994;15(5):399-409.

208. Fraser GE, Jacobs DR, Anderson JT, Foster N, Palta M, Blackburn H. The effect of various vegetable supplements on serum cholesterol. American Journal of Clinical Nutrition 1981;34:1272-1277.

209. Jenkins DJA, Popovich DG, Kendall CWC, Vidgen E, Tariq N, Ransom TPP, Wolever TMS, Vuksan V, Mehling CC, Boctor DL, Bolognesi C, Huang J, Patten R. Effect of a diet high in vegetables, fruit, and nuts on serum lipids. Metabolism 1997;46(5):530-537.

210. Katan MB. Direct and indirect effects of dietary fibre on plasma lipoproteins in man. Scandinavian Journal of Gastroenterology - Supplement 1987;129:218-222.

211. Kuo Y, Emmerling MR, Lampert HC, Hempelman SR, Kokjohn TA, Woods AS, Cotter RJ, Roher AE. High levels of circulating Aβ42 are sequestered by plasma proteins in Alzheimer's disease. Biochemical and Biophysical Research Communications 1999;257(787-791).

212. Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE, Selkoe SJ. Amyloid β-peptide is transported on lipoproteins and albumin in human plasma. Journal of Biological Chemistry 1996;271(51):32916-32922.

213. Koudinov A, Matsubara E, Frangione B, Ghiso J. The soluble form of Alzheimer's amyloid beta protein is complexed to high density lipoprotein 3 and very high density lipoprotein in normal human plasma. Biochemical and Biophysical Research Communications 1994;205(2):1164-1171.

214. Yoshitake T, Kiyohara Y, Kato I, Ohmura T, Iwamoto H, Nakayama K, Ohmori S, Nomiyama K, Kawano H, Ueda K, Sueishi K, Tsuneyoshi M, Fujishima M. Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly Japanese population: The Hisayama Study. Neurology 1995;45:1161-1168.

215. Broe GA, Henderson AS, Creasey H, McCusker E, Korten AE, Jorm AF, Longley W, Anthony JC. A case-control study of Alzheimer's disease in Australia. Neurology 1990;40:1698-1707.

216. Rogers RL, Meyer JS, Mortel KF. After reaching retirement age physical activity sustains cerebral perfusion and cognition. Journal of the American Geriatric Society 1990;38:123-128.

217. Crawford JG. Alzheimer's disease risk factors as related to cerebral blood flow: additional evidence. Medical Hypotheses 1998;50:25-36.

218. Mackic JB, Stins M, McComb JG, Calero M, Ghiso J, Kim KS, Yan SD, Stern D, Schmidt AM, Frangione B, Zlokovic BV. Human blood-brain barrier receptors for Alzheimer's amyloid-β 1-40: asymmetrical binding, endocytosis, and transcytosis at the apical side of brain microvascular endothelial cell monolayer. Journal of Clinical Investigation 1998;102:734-743.

219. Poduslo JF, Curran GL, Sanyal B, Selkoe DJ. Receptor-mediated transport of human amyloid β-protein 1-40 and 1-42 at the blood brain barrier. Neurobiology of Disease 1999;6:190-199.

220. Suzuki N, Iwatsubo T, Odaka A, Ishibashi Y, Kitada C, Ihara Y. High tissue content of soluble β1-40 is linked to cerebral amyloid angiopathy. American Journal of Pathology 1994;145(2):452-460.

221. Shinkai Y, Yoshimura M, Ito Y, Odaka A, Suzuki N, Yanagisawa K, Ihara Y. Amyloid β-proteins 1-40 and 1-42(43) in the soluble fraction of extra- and intracranial blood vessels. Annals of Neurology 1995;38(3):421-428.

222. Roher AE, Lowenson JD, Clarke S, Woods AS, Cotter RJ, Gowing E, Ball MJ. β-amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer's disease. Proceedings of the National Academy of Science USA 1993;90:10836-10840.

223. MicrowaveNews. EMF exposures: garment workers top utility employees. Microwave News 1995;15(5):6-7.

APPENDICES

Appendix A

Magnetic field-related exposure categories based on job title and industry (method #2).*

Definite/probable MF exposure
- Electric and telephone company: serviceman, lineman, and foreman
- Railroad and communication: engineer/technician
- Electrician
- Engineer/technician in electric, telecommunications, or aerospace industries
- Dispatcher
- Repairman in appliance and telecommunications industries
- Welder

Possible MF exposure
- Mechanic, machinist, repairman, maintenance man, stationary engineer, boilermaker
- Airline pilot, policeman, security guard
- Employees (not listed in above category) in utility, railroad, and telecommunications industry
- Engineers (not listed in above category)
- Steelworker, tool maker, plumber, pipefitter, carpenter
- Officer in Navy, Air Force, Coast Guard

No MF exposure
- All occupations other than those listed above

MF = magnetic fields

* Based on Lin RS, Dischinger PC, Conde J, Farrell KP. Occupational exposure to electromagnetic fields and the occurrence of brain tumors: an analysis of possible associations. J Occup Med 1985;276:413-419.

Appendix B

Adjusted odds ratios (and 95% confidence intervals) for each of the exposure methods, including education in the model[a]

	Alzheimer's disease	Amyotrophic lateral sclerosis	Parkinson's disease	Brain cancer
Method #1				
Electrical vs. non-electrical	0.96 (0.62, 1.48)	2.16 (1.14, 4.07)	1.54 (0.93, 2.55)	1.09 (0.66, 1.81)
Method #2				
Definite/Probable MF exposure	1.15 (0.77, 1.74)	1.45 (0.78, 2.70)	1.89 (1.20, 2.97)	1.00 (0.62, 1.61)
Possible MF exposure	1.19 (0.96, 1.47)	1.17 (0.80, 1.71)	1.16 (0.93, 1.44)	1.34 (1.00, 1.80)
No MF exposure (Referent)	1.00	1.00	1.00	1.00
Method #3				
1 (Highest quartile)	0.93 (0.70, 1.25)	0.87 (0.53, 1.41)	1.27 (0.95, 1.71)	1.30 (0.87, 1.94)
2	1.12 (0.84, 1.49)	0.66 (0.41, 1.07)	1.32 (1.01, 1.74)	0.84 (0.58, 1.23)
3	1.12 (0.84, 1.49)	0.66 (0.41, 1.07)	1.32 (1.01, 1.74)	0.84 (0.58, 1.23)
4 (Referent)	1.00	1.00	1.00	1.00

[a] Adjusted for education (>=12 years/< 12 years) race (white/non-white) and social class (managerial/professional, technical/sales/administrative support, service/farming/craft/repair/operators/ fabricators/laborers). Two years of data missing due to lack of education variable.

MF = magnetic field.

Appendix C

CONSENT FORM

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Melatonin and Occupational EMF Exposure

NAME OF PRINCIPAL INVESTIGATOR: John S. Reif, D.V.M.
Professor and Head
Department of Environmental Health
Colorado State Univeristy
Fort Collins, CO 80532

NAME OF CO-INVESTIGATOR: James Burch, M.S., Ph.D.
Research Associate
Department of Environmental Health
Colorado State Univeristy
Fort Collins, CO 80532

CONTACT NAMES AND PHONE NUMBERS FOR QUESTIONS/PROBLEMS:

Dr. John Reif
PHONE NO.: (970) 491-6074

Dr. James Burch
PHONE NO.: (970) 491-6178/8612

SPONSOR OF PROJECT: National Insititute of Environmental Health Sciences/U.S. Department of Energy/Platte River Power Authority

PURPOSE OF THE RESEARCH: The purpose of this study is to investigate how magnetic fields may affect the body and what biological processes are involved. We are conducting a study to evaluate whether exposure to magnetic fields has an effect on the production of the hormone, melatonin. We wish to determine whether melatonin production is related to a person's moods. We also want to study several biological parameters in blood that may be associated with magnetic field exposures and with melatonin production including immune cell and beta amyloid concentrations and ornithine decarboxylase activity (ODC). Beta amyloid is a protein found in all people, and deposits of this protein are found in Alzheimer's patients. ODC is an enzyme found in all cells, and its level of activity indicates cell growth. Melatonin, ODC and beta amyloid measurements have no medical implications. Changes in these parameters may help explain how magnetic fields affect the body.

PROCEDURES/METHODS TO BE USED: The basic procedures to be used in this study will be to monitor the magnetic fields that you are normally exposed to and then measure your production of melatonin over a period of four days. Magnetic fields will be monitored using a meter in a belt pack that you will wear. Melatonin levels will be measured by analyzing the amount of a melatonin by-product (e.g. 6-OHMS or AMK) that you normally pass in your urine. You will be asked to collect two urine samples per day for four days, one in the morning immediately after you wake up and one at the end of your work shift. Information obtained from this study will be strictly confidential. Your individual melatonin and blood sample results will be provided to you upon request. We will store a portion of your sample for further analysis at a later date. This sample may be analyzed for testosterone, estrogen or other hormones or proteins that may be affected by changes in melatonin.

We ask that you perform your duties as you would under normal circumstances and that you wear the meter at all times during the study period while at work and at home. When asleep, we ask that you place the meter by your bed. We will provide you with a timecard to write down the times that you begin and end each work shift, arrive at home after work, go to bed, and wake up.

We would like you to repeat this 3-day sampling process three more times over a 1 year period. There will be a space of about 3 months between each 3-day data sampling period. At the end of one study period, we would like you to answer a series of questions about your health, diet (including alcohol and tobacco use), current and past occupations and daily activities. We will also ask you some questions about your moods. This will take about 20 to 30 minutes. At the end of the other study periods, we will ask you to complete a shorter, 15-30 minute questionnaire about your activities.

As a participant in this study, you will be asked to provide one blood sample (about 2-3 tablespoons). Your sample will be collected by a nurse at the end of one study period. Your estimated time requirement for blood sample collection is 15 minutes. There is a possibility that we will contact you again to participate in a further study of magnetic fields and melatonin. If so, we will again obtain your written consent and your participation will be voluntary, as it is now.

RISKS INHERENT IN THE PROCEDURE: No significant risks are anticipated from participation in this study. No additional hazard or physical stress is expected from wearing the meter. The weight of this device is similar to that of tool or other instruments that you might typically carry. The possibility exists that you may find certain questions from the questionnaire objectionable. Completion of the questionnaire is voluntary. If you do not wish to answer any of the questions in the questionnaire, you may skip them. Risks associated with blood sampling include a possible bruise, slight risk of infection, local soreness, and fainting. A nurse will collect your sample and precautions will be taken to ensure your safety.

BENEFITS: Electric utility workers have EMF exposures that are greater, on the average, than other occupations. Information obtained from this study will increase our understanding of the biological effects of magnetic fields. If there are health effects associated with exposure to EMFs, you may benefit from exposure limits that could be developed based on the information obtained from this study. The analysis for immune parameters in your blood sample will be reviewed to see if they are within normal ranges. If your results are outside normal ranges, you will be contacted by letter and advised to contact your personal physician. Because we are asking you to wear a meter for 24 hours each day, including your leisure time, and we are asking you to keep a log of your activities, we will reimburse you $25 for each week that you participate in this study. If you participate in blood sample collection, you will receive an addiational $50.

CONFIDENTIALITY: The results of your health interview, magnetic field measurements, urine and blood tests will be kept strictly confidential. All records will be kept locked at the Colorado State University Department of Environmental Health, accessible only to authorized personnel. Your name or results will not be released to any private party, employer, or insurance company. The results of this study will be released as group data only. There will be no means of identifying you personally in any results that are published.

LIMITATION OF LIABILITY: The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

Questions concerning your rights as a study participant may be addressed to Celia Walker, Human Research Committee Administrator, at 970-491-1563.

PARTICIPATION: I understand that it is not possible to identify all potential risks in a study of this type, but I believe that reasonable safeguards have been taken to minimize both the known and the potential, but unknown, risks.

I understand that my participation in this research is voluntary. If I decide to participate in the study, I may withdraw my consent and stop participating at any time without penalty or loss of benefits to which I am otherwise entitled.

I have read and understand the information stated and willingly sign this consent form. My signature also acknowledges that I have received, on the date signed, a copy of this document containing 3 pages.

I _____(name of the researcher obtaining consent) have discussed the above information with _____(participant's name) and have addressed questions to his/her satisfaction.

_____	_____	_____am/pm
Signature of person obtaining consent	Date	Time
_____		
Participant name (printed)		
_____	_____	_____am/pm
Participant's signature	Date	Time

This form was approved for use by the CSU Human Research Committee for up to 12 months from the approval date, May 7, 1998. (Approval 95-282H)

Appendix D

Power Calculations

There are currently no animal or human data on which to base estimates of change in circulating Abeta levels due to magnetic field exposure. Elevated levels of plasma Abeta that could be considered risk factors for Alzheimer's disease are those found among presymptomatic individuals with a familial Alzheimer's disease mutation on the amyloid precursor protein (APP) gene. Presymptomatic carriers of the APP717 mutation had 41% higher levels of plasma Abeta(1-42/3).[1] Down's Syndrome patients prior to the presentation of dementia had 85% higher levels of plasma Abeta(1-42/3).[2]

For the purposes of power calculations, the distribution and generation workers are considered the exposed group and administration and maintenance workers are considered the unexposed group. The statistical power to detect a difference in the means of circulating Abeta between the exposed and unexposed groups is a function of the expected difference, the standard deviation, the number of subjects per group, and the level of significance. The power calculations presented in Table D.1 are based on 20% to 50% differences from a given

---

1 Kosaka T, Imagawa M, Seki K, Arai H, Sasaki H, Tsuji S, Asami-Odaka A, Fukushima T, Imai K, Iwatsubo T. The bAPP717 Alzheimer mutation increases the percentage of plasma amyloid-b protein ending at Ab42(3). Neurology 1997;48:741-745.

2 Tokuda T, Fukushima T, Ikeda S, Sekijima Y, Shoji S, Yanagisawa N, Tamaoka A. Plasma levels of amyloid b proteins Ab1-40 and Ab1-42(3) are elevated in Down's Syndrome. Annals of Neurology 1997;41:271-273.

level of circulating Abeta. The baseline mean and standard deviation (10.7 ± 2.1 pM) are taken from a study of circulating Abeta (1-42/3) in a non-demented control population (n = 15).[1] We used a power chart to determine the power function of a two-sided t test based on calculated values for ϕ and ν,3 given by:

$$\phi = (\delta \sqrt{n}) / (\sigma * 2),$$

where δ = difference in means between treatment groups,
n = number of subjects in each treatment group,
σ = the standard deviation of the mean (e.g., 2.1); and

$$\nu = 2 * (n - 1).$$

Table D.1: Power to detect a 20% - 50% change in circulating Abeta(1-42/3) at the α = .05 level of significance.

n (per treat.)	20%	30%	40%	50%
10	.56	.90	.99	>.99
15	.78	.98	>.99	>.99
20	.89	>.99	>.99	>.99
25	.94	>.99	>.99	>.99
30	.97	>.99	>.99	>.99

Table D.1 indicates that a sample size of 20 per treatment group, or a total of 40 subjects, will provide adequate power to detect a 20% to 50% change in circulating Abeta.

The current study was based on the power calculations presented above. The mean and standard deviation upon which these values are based, however, refer to a population that is older (mean age = 72 years)[1] relative to our study population (mean age = 41 years). After looking at the distribution of Abeta values among our study

---

3 Pearson, E.S. and Hartley, H.O. (Eds.) Biometrika Tables for Statisticians Vol. 1. Cambridge University Press. New York, NY. 1970.

population, the following power calculations were generated for the three Abeta parameters (Tables D.2 – D.4). Although we had adequate power in this study to detect 30% changes in Abeta(1-40), we did not have adequate power to detect changes in Abeta(1-42) or the ratio of Abeta(1-42) to Abeta(1-40).

Table D.2: Power to detect a 20% - 50% change in circulating Abeta(1-40) the α = .05 level of significance.

n (per treat.)	20%	30%	40%	50%
30	.60	.92	>.99	>.99
40	.76	.97	>.99	>.99
50	.80	>.99	>.99	>.99

Table D.3: Power to detect a 20% - 50% change in circulating Abeta(1-42) at the α = .05 level of significance.

n (per treat.)	20%	30%	40%	50%
30	<.40	<.40	<.40	.55
40	<.40	<.40	.50	.65
50	<.40	<.40	.55	.76

Table D.4: Power to detect a 20% - 50% change in the Abeta ratio (Abeta(1-42)/Abeta(1-40)) at the α = .05 level of significance.

n (per treat.)	20%	30%	40%	50%
30	<.40	<.40	.55	.74
40	<.40	.42	.65	.80
50	<.40	.50	.76	.92

Appendix E

Sample Log and Time Log

MELATONIN LEVELS IN ELECTRIC UTILITY WORKERS

CSU Subject ID Number:_____ Start Date: _____
Employer: _____

SAMPLE COLLECTION LOG

Sample Number	Sample Date	Time of Urine Collection (Circle one)
		Morning Afternoon
		Morning Afternoon
		Morning Afternoon
		Morning Afternoon
		Morning Afternoon
		Morning Afternoon
		Morning Afternoon

TIME LOG

Day	Time awoke	Left for work	Time arrived at work	Time left work	Time arrived at home	Time went to sleep
Sun.					START=>	
Mon.						
Tue.						
Wed.						
Thu.		<=STOP				

Appendix F

Questionnaire

MELATONIN AND OCCUPATIONAL MAGNETIC FIELD EXPOSURE

(Follow-up, Round C)

PERSONAL DATA SHEET

********** **PLEASE PRINT**********

Date: _____

CSU Identification Number: _____ (Note: Your CSU ID number will be assigned by a CSU researcher).

Subject Name:

_____

(Please Print)

Please fill out the information below if it has changed since the last time you participated in this study.

Home Address: _____

City: _____ State: ____ Zip: _____

Home Phone: ( ) -_____

Work Phone: ( ) -_____

Social Security No.: _____

CSU Identification Number:_____

MELATONIN AND OCCUPATIONAL MAGNETIC FIELD EXPOSURE

FOLLOW-UP QUESTIONNAIRE (Round C)

INSTRUCTIONS: Answer each question by circling the answer or by filling in the blank. If you have a question, please ask the CSU representative who is administering this questionnaire. If you choose not to answer a question, please write "R" after the question.

THANK YOU FOR YOUR TIME AND COOPERATION.

1.0 OCCUPATION

1.1 Has your job title changed since the last time you participated in this study?

[1] Yes
[2] No

If yes, enter new title:_____

1.2 Were you called in to work after 6:00 p.m.:

a. on the days you participated in this study?

	1st Work Day	2nd Work Day	3rd Work Day
	[1] Yes [2] No	[1] Yes [2] No	[1] Yes [2] No
Approximate amount of time (hours)	_____hrs.	_____hrs.	_____hrs.

b. the week before you participated in this study (at least 1 day)?

[1] Yes
[2] No

1.3 Within the last 2 weeks, how would you best describe the amount of physical activity in your work, on the average?

[1] extreme (physically demanding on a daily basis)
[2] high (physical exertion several days per week)
[3] moderate (physical exertion less than once per week)
[4] mild (little or no physical exertion)

1.4 DID YOU CONDUCT ANY OF THE FOLLOWING ACTIVITIES **AT WORK ON THE DAYS THAT YOU PARTICIPATED IN THIS STUDY**:

a. Work outdoors?

	1st Work Day	2nd Work Day	3rd Work Day
	[1] Yes [2] No	[1] Yes [2] No	[1] Yes [2] No
Approximate amount of time (hours)	_____hrs.	_____hrs.	_____hrs.

b. Conduct strenuous physical work (for at least 30 minutes)?

	1st Work Day	2nd Work Day	3rd Work Day
	[1] Yes [2] No	[1] Yes [2] No	[1] Yes [2] No
Approximate amount of time (hours)	_____hrs.	_____hrs.	_____hrs.

1.5 **WITHIN THE LAST 2 WEEKS**, HAVE YOU REGULARLY HANDLED OR COME INTO CONTACT WITH ANY OF THE FOLLOWING **AT WORK OR AT HOME**:

a. Insecticides, pesticides, or herbicides?

[1] yes
[2] no

If yes, please list:

_____

_____

Question 1.5 Continued: WITHIN THE LAST 2 WEEKS, HAVE YOU REGULARLY HANDLED OR COME INTO CONTACT WITH ANY OF THE FOLLOWING **AT WORK OR AT HOME**:

b. Old transformers or PCB's (polychlorinated biphenyls, which are found in old transformer oils)?

[1] yes
[2] no

c. Fuels, solvents, or degreasers?

[1] yes
[2] no

If yes, please list the compound(s) used:

_____

_____

d. Creosote (e.g., treated utility poles)?

[1] yes
[2] no

e. Any other chemical, hazardous material, or hazardous waste?

[1] yes
[2] no

If yes, please specify:

_____

_____

2.0 LIFESTYLE

2.1 Have you changed your smoking habits since the last time you participated in this study?

[1] yes
[2] no

If no, skip to question 2.4

2.2 Do you currently smoke cigarettes?

[1] yes
[2] no

2.3 If yes, how many **packs** of cigarettes do you currently smoke **each day**, on the average (1 pack = 20 cigarettes)?

Number of Packs per Day: _____
(Enter 0 if you are a nonsmoker)

2.4 Are you exposed to the smoke from other people's cigarettes, pipes, or cigars on a daily basis while at home or at work?
(Circle all that apply).

[1] yes, at home only
[2] yes, at work only
[3] yes, at home and at work
[4] no

2.5 Have you changed your alcohol consumption habits since the last time you participated in this study?

[1] yes
[2] no

If no, skip to question 2.9

2.6 Do you drink alcoholic beverages?

[1] yes
[2] no

2.7 How many **days per week** do you drink alcoholic beverages, on the average?

Days per week: _____ (Enter zero if none).

2.8 About how many drinks do you consume on days when you drink, on the average? (Note: A drink is 1 can or bottle of beer, or 1 glass of wine or wine cooler, or 1 cocktail, or 1 shot of liquor).

Number of drinks _____ (Enter zero if none).

2.9 How many **caffeinated beverages** do you drink **per day**, on the average? (Enter 0 if none).

[1] Coffee: _____
[2] Tea: _____
[3] Chocolate (Cocoa): _____
[4] Soda Pop (Soft Drinks, Cola): _____
[5] Other: _____ (Specify): _____
[9] Don't Know

2.10 Do you take any of the following supplements on a regular basis?

a. Multiple vitamin

[1] yes
[2] no

b. Vitamin C

[1] yes
[2] no

c. Vitamin E

[1] yes
[2] no

d. Beta-carotine

[1] yes
[2] no

e. Other (specify): _____

2.11 Rate the frequency with which you consume the following food items.

a. Vegetables

[1] 4 or more servings per day
[2] 2 to 3 servings per day
[3] One serving per day
[4] Occasionally (Less than one serving per day)
[5] Never

b. Fruits

[1] 4 or more servings per day
[2] 2 to 3 servings per day
[3] One serving per day
[4] Occasionally (Less than one serving per day)
[5] Never

2.12 Did you exercise for **at least 30 minutes on the days that you participated in this study**?

	1st Work Day	2nd Work Day	3rd Work Day
	[1] Yes [2] No	[1] Yes [2] No	[1] Yes [2] No
Approximate amount of time (hours)	_____hrs.	_____hrs.	_____hrs.

3.0 MEDICAL

3.1 Are you currently taking any of the following medications **on a daily basis** or **as prescribed by your physician**?

a. Melatonin?

[1] yes
[2] no

b. Anabolic steroids or other muscle builders (for example,
DHEA, IGF, growth hormone, etc.)

[1] yes
[2] no

c. Cortisol (Cortisone, Hydrocortisone)?

[1] yes
[2] no

d. Aspirin?

[1] yes
[2] no

e. Acetaminophen (Tylenol)?

[1] yes
[2] no

f. Ibuprofen (Advil, Motrin)?

[1] yes
[2] no

g. Tranquilizers or Sleeping Pills (e.g., Valium, Ativan,
Xanax, Klonopin, Restoril, Dalmane or other)?

[1] yes
[2] no

h. Antidepressants (Prozac, Zoloft, Paxil, Effexor,
Trazodone, Luvox, Serzone or other)?

[1] yes
[2] no

Question 3.1 Continued: Are you currently taking any of the following medications **on a daily basis** or **as prescribed by your physician**?

i. Prescription Eye Drops?

[1] yes
[2] no

j. Prescription Pain medication?

[1] yes
[2] no

k. Herbal or Homeopathic medicines?

[1] yes
[2] no

l. Over-the-Counter Cold Remedies?

[1] yes
[2] no

3.2 Are you currently taking medication for any of the following diseases **on a daily basis** or **as prescribed by your physician:**

a. Psychiatric disorder (neurosis or psychosis)?

[1] yes
[2] no

If yes, please enter name(s): _____

b. Skin disorder (for example, psoriasis)?

[1] yes
[2] no

If yes, please enter name(s): _____

c. Heart disease or a cardiovascular disorder?

[1] yes
[2] no

If yes, please enter name(s): _____

Question 3.2 Continued: Are you currently taking medication for any of the following diseases on a daily basis or **as prescribed by your physician:**

d. High blood pressure or hypertension?

[1] yes
[2] no

If yes, please enter name(s): _____

e. Asthma?

[1] yes
[2] no

If yes, please enter name(s): _____

f. Any other medication?

[1] yes
[2] no

If yes, please enter name(s): _____

3.3 Has one of your direct (blood) relatives had any of the following diseases?

a. Alzheimer's disease

[1] yes
[2] no

b. Parkinson's disease

[1] yes
[2] no

c. Down's syndrome

[1] yes
[2] no

d. Amyotrophic lateral sclerosis (Lou Gehrig's disease)

[1] yes
[2] no

3.4 Have you ever had a head injury which required medical care and/or resulted in loss of consciousness?

[1] yes
[2] no

3.5 If your head injury resulted in a loss of consciousness, please indicate the year in which the injury occurred: _____

4.0 ALUMINUM

The following questions are designed to assess potential factors that may influence your exposure to aluminum.

4.1 Do you currently take antacids on a daily basis or as prescribed by your physician?

[1] yes
[2] no

If yes, please specify brand name:_____

4.2 Do you use antiperspirants or deodorants on a daily basis?

Antiperspirants?

[1] yes If yes, please specify brand name: _____
[2] no

Deodorants?

[1] yes If yes, please specify brand name: _____
[2] no

4.3 Do you use aluminum containing cookware (pots and pans) at home?

[1] yes
[2] no

4.4 What proportion of your daily water consumption comes from bottled water?

[1] 0
[2] less than ¼
[3] ¼ to ½
[4] ½ to ¾
[5] more than ¾

If more than 0, please specify brand name:_____

Appendix G

Determination of sample preservation and collection procedures.

A previous study using enzyme-linked immunosorbent assay (ELISA) procedures for the detection of Abeta in human plasma used preservatives when collecting blood samples (Tamaoka96), while another did not use any preservative other than the EDTA contained in the collection tube.(Mehta98) Moreover, these previous studies were conducted under controlled laboratory conditions, whereas we would be collecting samples in the field and transporting them to the laboratory for processing. Thus, it was imperative to determine the optimum means for collection, preservation, and processing of blood samples for Abeta analysis. Prior to conducting our study, blood samples were obtained from three volunteers to observe the effects of different methods of preservation and processing. These plasma samples were sent overnight on dry ice to the laboratory of Dr. Pankaj Mehta of the New York State Institute for Basic Research, Department of Immunology.

Preliminary findings suggested the optimum means for collection, preservation, and processing of blood samples for Abeta analysis. Figure F presents Abeta results from 3 subjects, using different methods of sample preservation and different holding times prior to processing. Based on these data, the use of preservative did not appear to have a consistently beneficial or detrimental effect. An analysis of variance (ANOVA) indicated no significant difference in Abeta(1-40) or Abeta(1-42) between preserved and

unpreserved samples ($p = 0.69$ and 0.10, respectively). Similarly, there was no indication of significant variability in Abeta(1-40) or Abeta(1-42) by different periods of time delay before processing ($p = 0.99$ and 0.86, respectively) or by the interaction of holding time and sample preservation ($p = 0.75$ and 0.91, respectively). While Abeta(1-40) levels were relatively stable over different holding periods, however, two of the three subjects demonstrated high variability in the detection of Abeta(1-42) levels over time. These findings suggested the importance of consistency in the handling of samples. Based on these results, no preservative was added; and, as indicated in the Methods section, all blood samples for Abeta analysis were held for the same specified time period prior to processing and storage.

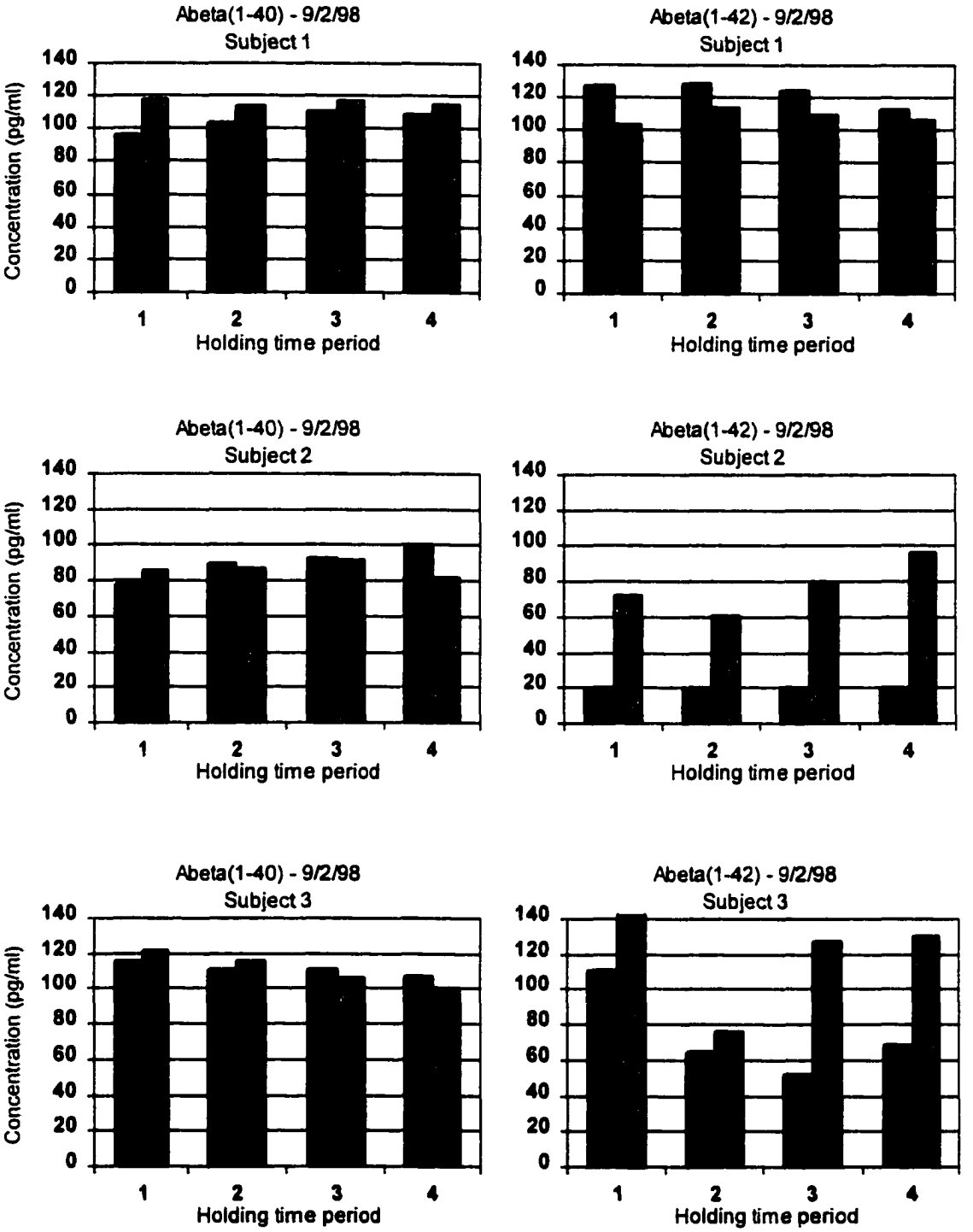

Figure G. Comparison of Abeta results for preserved and unpreserved blood samples over different time periods. Dark bars represent samples preserved with sodium azide and phenylmethylsulfonyl flouride, added immediately following blood draw. Light bars represent unpreserved samples. Time period 1 indicates samples that were immediately centrifuged. Remaining samples were held on ice and processed at one hour time intervals, time periods 2-4. Abeta(1-42) levels for Subject 2 were below detection limits.

Appendix H

Descriptive Abeta results of control samples.

One control blood sample was drawn from the same individual during each of the sampling events. This individual was not a participant in the MF study and does not have any corresponding MF exposure or melatonin data. These control samples were handled and processed by the same procedures described above.

Table H. Descriptive statistics of Abeta parameters among control samples.

	Abeta(1-40)	Abeta(1=42)	Abeta Ratio Abeta(1-42)/Abeta(1-40)
Number of samples	13	13	13
Mean	78.15	79.9	1.00
Standard Deviation	9.8	47.1	0.56
Minimum value	61.8	4.9	0.08
Maximum value	88.1	193.6	2.34

Appendix I

Procedures and solution used for enzyme-linked immunosorbent assay (ELISA) procedure to quantify Abeta in plasma.

ELISA procedure developed by Dr. Pankaj Mehta, Department of Immunology, New York Institute for Basic Research, Staten Island, NY.

I. Equipment

1. ELISA plates: U96 Maxisorp NUNC Immunoplates
2. Plate Covers: Falcon 3913 Flexible lid microtest III

II. Solutions and General Methods

1. Coating buffer = $Na_2Co_3/NaHCO_3$ 0.05 M

Solution A: Na_2Co_3 0.05M
1000 ml 1M = 105.99 gm
100 ml 0.05M = 0.529 gm

Soluction B: $NaHCO_3$ 0.05M
1000 ml 1M = 84.01 gm
100 ml 0.05M = 0.42 gm

Add solution A to solution B to get pH = 9.6

Coating buffer is good for up to two weeks if stored at 4°C.
Coated plates are good for up to two month if stored at -20°C.

2. PBS (phosphate buffered saline) 0.85% NaCl

Solution A: $NaH_2PO_4 \cdot H_2O$ 0.01M (sodium phosphate monobasic)
1000 ml 1M = 137.99 gm
1000 ml 0.01M = 1.379 gm
500 ml 0.01M = 0.689 gm
Add 4.25 gm NaCl

Solution B: Na_2HPO_4 0.01M (sodium phosphate dibasic)
1000 ml 1M = 141.96 gm
1000 ml 0.01M = 1.42 gm
Add 8.5 gm NaCl

Add solution A to solution B to get pH = 7.2

3. PBST
PBS + 0.05% Tween-20
100 ml PBS + 0.5 ml Tween-20
4. Diluent buffer/Blocking buffer
PBST + 1% BSA (bovine serum albumin)
100 ml PBST + 1 gm BSA

Diluent buffer must be prepared fresh every day.

5. Standards (STD) preparation
Abeta(1-40) from Bachem 1mg/ml in Hexafluroisopropanol
Abeta(1-42) from Bachem 1 mg/ml in Dimethylsulfoxide
Put STDs in 8 ul aliquot before freezing.
Store STDs at -80°C.
Prepare dilutions using diluent buffer (PBST + 1% BSA)

5 ul STD in 5 ml diluent buffer = 1000 ng/ml
0.1 ml 1000ng/ml in 10 ml diluent buffer = 10 ng/ml
1 ml 10 ng/ml + 1 ml diluent buffer = 5 ng/ml
1 ml 5 ng/ml + 1 ml diluent buffer = 2.5 ng/ml
1 ml 2.5 ng/ml + 1 ml diluent buffer = 1250 pg/ml
Repeat to get the following concentrations:
1250 pg/ml
625 pg/ml
312.5 pg/ml
156.25 pg/ml
78.125 pg/ml
39.063 pg/ml
19.531 pg/ml
9.766 pg/ml
4.883 pg/ml

For Abeta(1-40) use 8 STDs: 4.883 pg/ml – 626 pg/ml
For Abeta(1-42) use 9 STDs: 4.883 pg/ml – 1250 pg/ml

6. Washing plates
   Use PBST for washing
   275-300 ul/well
   15-20 sec. wait between each wash
   After wash, hit plate on blotter to remove excess

7. Polyclonal Antibodies (affinity purified)
   R162 Biotinylated Abeta(1-40)
   R165 Biotinylated Abeta(1-42)
   Keep at -20°C.

III. Method

1. Coating of plates with monoclonal Abeta (6E10).
   2.5 ug/ml 6E10 in coating buffer at pH 9.6
   Coating buffer = $Na_2Co_3/NaHCO_3$ 0.05 M
   Add 100 ul/well of 6E10/coating buffer solution.
   Suggested not to use outer wells of plate because they tend not to absorb properly.
   Store plates at 4°C overnight.
   Wash plates next day 4 X with PBST

   Coated plates can be stored at 4°C for one month after washing.

2. Add blocking buffer
   200 ul/well 1% BSA in PBST
   Let plates stand 1 hour at room temperature (with covers)
   Wash plates 4 X with PBST

3. Add standards and samples.
   Each sample will be placed on an Abeta(40) and an Abeta(42) plate.
   Add STDs in rows 6, 7 of plate, and duplicate STDs in rows 10, 11.
   8 STDs for Abeta(40) plate and 9 STDs for Abeta(42) plate.
   Add samples undiluted.
   100 ul/well for both STDs and samples.
   Let plates stand 2 hours at room temperature.
   Place in 4°C overnight.
   Wash plates next day 4 X with PBST

4. Add biotinylated antibodies.
   Add Antibodies immediately after washing plates, do not let plates dry out.

   R162 diluted at 1:1000
   10 ul R162 in PBST + 1%BSA
   Let solution stand 15 minutes before adding to allow biotin to go in solution.
   100 ul/well

   R165 diluted at 1:400
   10 ul R165 in PBST +1%BSA
   Let solution stand 15 minutes before adding to allow biotin to go in solution.
   100 ul/well

   Let plates stand 1:30 hour at room temperature.

   Wash plates 4 X with PBST

5. Add Neutravidin HRP conjugated 1 mg/ml (Pierce)
   5 ul of 1 mg/ml in 5 ml PBST (1:1000 dilution)
   Let solution stand 30 minutes
   1 ml of 1:1000 solution in 10 ml PBST (1:10,000 dilution)
   Let solution stand 30 minutes
   100 ul/well

   Let plates stand 1 hour at room temperature.

   Wash plates 4 X with PBST

6. Add Substrate buffer
   TMB Microwell Peroxidase Substrate system (Kirkegaard and Perry Lab, Gaithersberg, MD)
   Mix equal volumes of bottles A and B just before using.
   100 ul/well
   Also put 100 ul in top left well (empty well at this point) to subtract out optical density of substrate.

7. Add Stop solution.
   1 M phosphoric acid
   100 ul/well
   For R162, add stop solution 15 minutes after adding substrate
   For R165, add stop solution 25 minutes after adding substrate

8. Read plates.
   450 nm

IV. Graphing

Standards are their respective optical densities were graphed to a 3rd order polynomial using Cricket 1.3 software. The resulting formula was used to convert opitical densities to concentrations. An example of a standard concentration graph for a typical ELISA plate is shown below.

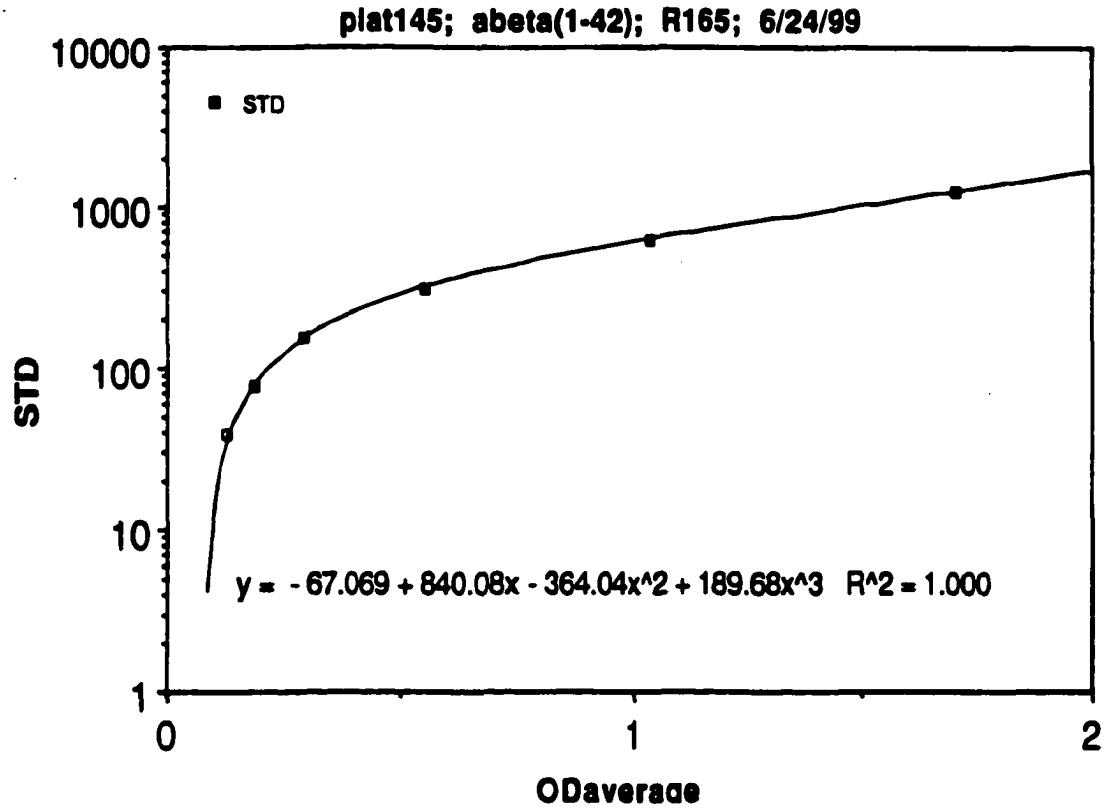

Figure I. Sample standard curve from ELISA plate.

STD = standard; ODaverage = average optical density for standards in duplicate.
Note – Standard axis is in log scale.

Appendix J

Transformation of dependent variables.

We used the Box-Cox procedure in Minitab 12.1 to establish the best transformation to achieve homogeneity of variance. For each of the dependent variables, the subgroup used was a four category combination of vegetable consumption and income. The lambda estimates for each of the dependent variables suggests a square-root transformation (see next page). Square-root transformation also achieve normality when univariately evaluating the residuals of regression and analysis of variance models.

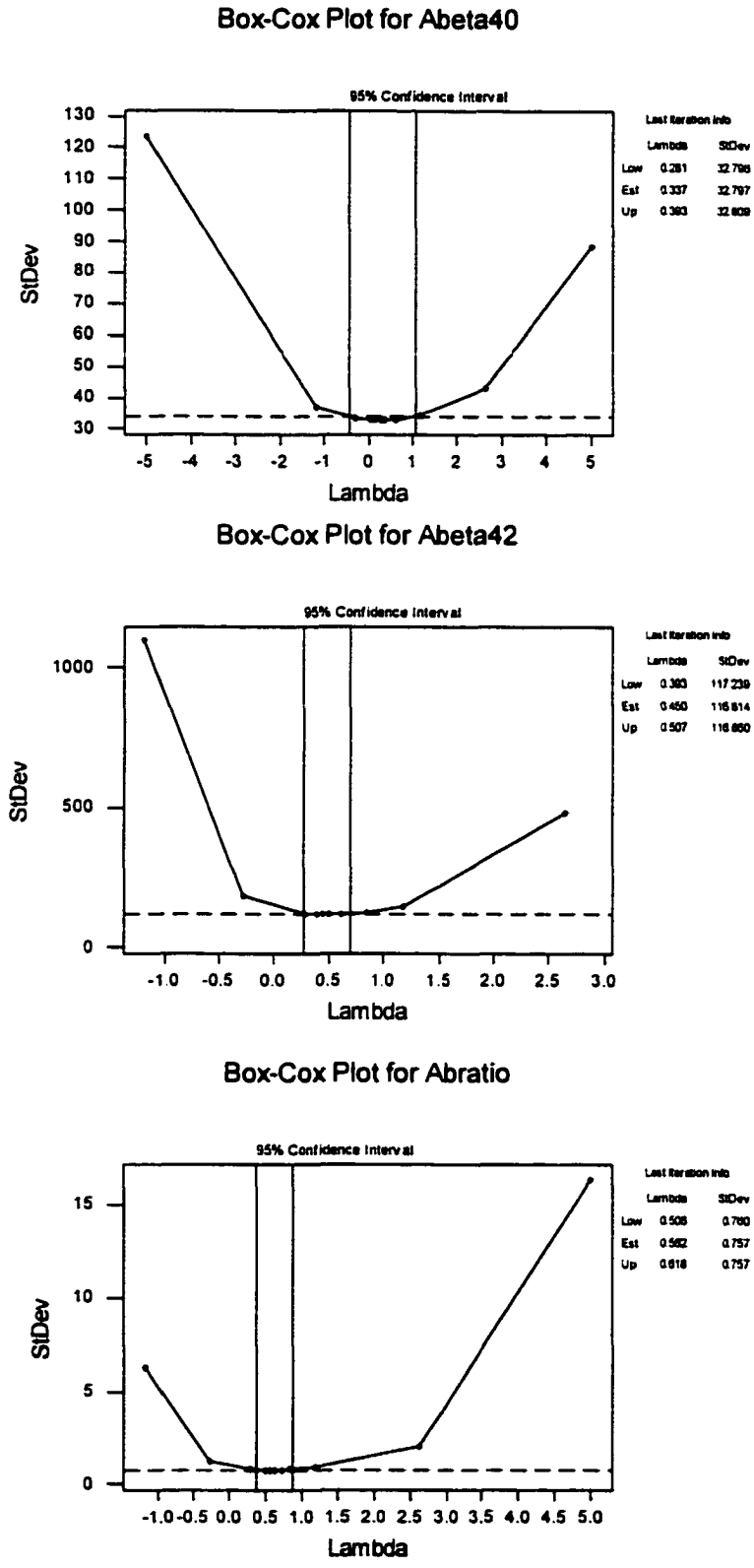

Figure J. Box-Cox plots for Abeta parameters.

Appendix K

Summary of magnetic field exposures for study population

Table K.1. Magnetic field exposure metrics for the two hours prior to the subjects' blood draw.

Exposure metric	Distribution/ Generation workers (n = 44)	Administration/ maintenance workers (n = 16)	Total (n = 60)
Arithmetic TWA (mG)	3.67	1.03	2.97
Geometric TWA (mG)	1.31	0.62 *	2.34
Cumulative Exposure (mG-hrs.)	7.34	2.06	5.94
Cumulative Exposure > 2 mG (mG-hrs.)	6.40	1.07	4.98
RCM (mG/15 seconds)	3.27	0.88	2.63
RCMS (per 15 seconds)	0.79	0.81	0.79

TWA = time weighted average; mG = milliGauss; RCM = rate of change metric; RCMS = standardized rate of change metric.

* $p < 0.05$ for T-test of the difference between group means. Exposure metrics (except RCMS) were log-transformed for T-test to satisfy assumption of normality.

Table K.2. Magnetic field exposure metrics for the work period averaged over three days.[a]

Exposure metric	Distribution/ Generation workers (n = 44)	Administration/ maintenance workers (n = 16)	Total (n = 60)
Arithmetic TWA (mG)	2.86	0.86 *	2.32
Geometric TWA (mG)	0.95	0.50	0.83
Cumulative Exposure (mG-hrs.)	78.68	22.43 *	63.68
Cumulative Exposure > 2 mG (mG-hrs.)	64.21	10.83	49.98
RCM (mG/15 seconds)	5.47	1.46	4.40
RCMS (per 15 seconds)	0.70	0.78	0.72

[a] Only two days of work magnetic field exposure for one subject.
TWA = time weighted average; mG = milliGauss; RCM = rate of change metric; RCMS = standardized rate of change metric.
* $p < 0.05$ for T-test of the difference between group means. Exposure metrics (except RCMS) were log-transformed for T-test to satisfy assumption of normality.

Appendix L

Association between physical work and Abeta

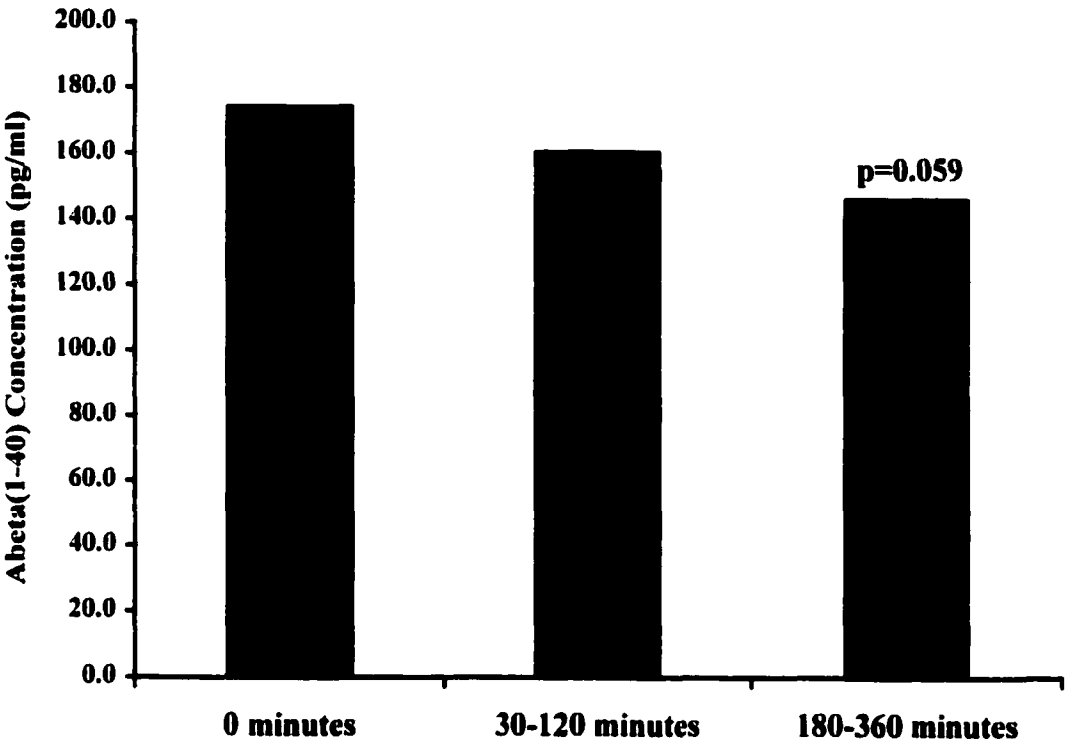

Figure L.1. Abeta(1-40) Least Square Means by Level of Physical Work on the Day of the Blood Draw.[a]

[a] Means are adjusted for age, income, and vegetable consumption.
p-value is for comparison of the difference between means of the 3rd and 1st tertiles.

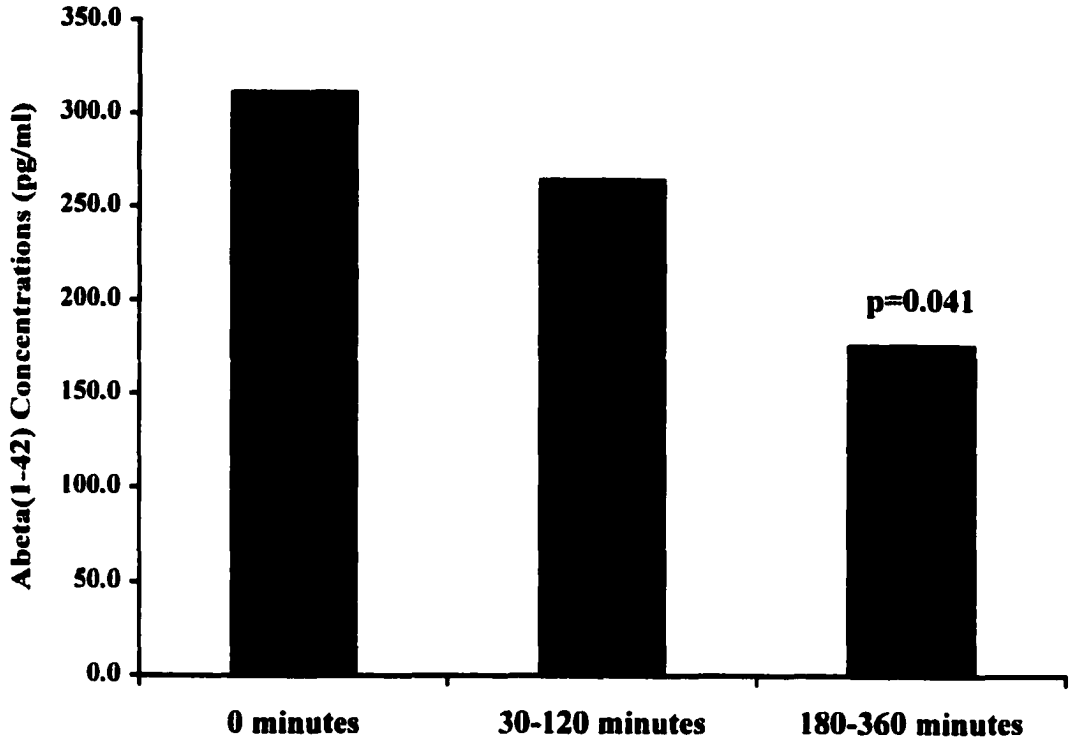

Figure L.2. Abeta(1-42) Least-squares Means by Level of Physical Work on the Day of the Blood Draw.[a]

[a] Means are adjusted for age, income, and vegetable consumption.
p-value is for comparison of the difference between means of the 3rd and 1st tertiles.

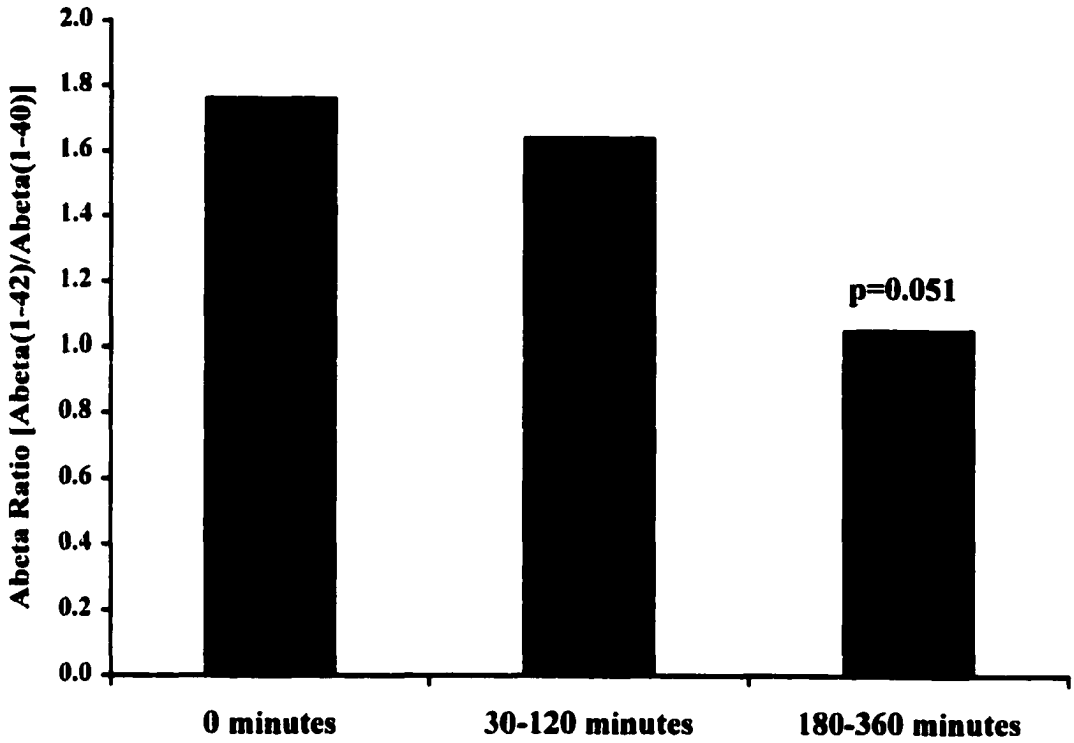

Figure L.3. Abeta Ratio [Abeta(1-42)/Abeta(1-40)] Least-squares Means by Level of Physical Work on the Day of the Blood Draw.[a]

[a] Means are adjusted for age, income, and vegetable consumption.
p-value is for comparison of the difference between means of 3rd and 1st tertiles.

Appendix M

Correlation between magnetic field exposure and Abeta

Table M.1. Spearman's correlation coefficients and p-values for Abeta variables versus magnetic field exposure metrics for work on the day of the subjects' blood draw.[a]

Exposure metric	Abeta(1-40)	Abeta(1-42)	Abeta Ratio Abeta(1-42)/ Abeta(1-40)
Arithmetic TWA (mG)	-0.04 (p = 0.77)	-0.05 (p = 0.70)	-0.10 (p = 0.43)
Geometric TWA (mG)	0.13 (p = 0.34)	0.12 (p = 0.35)	0.07 (p = 0.59)
Cumulative Exposure (mG-hrs.)	0.01 (p = 0.95)	-0.04 (p = 0.79)	-0.09 (p = 0.50)
Cumulative Exposure > 2 mG (mG-hrs.)	-0.07 (p = 0.62)	-0.10 (p = 0.44)	-0.16 (p = 0.24)
RCM (mG/15 seconds)	-0.11 (p = 0.42)	-0.21 (p = 0.11)	-0.27 (p = 0.04)
RCMS (per 15 seconds)	0.03 (p = 0.79)	-0.08 (p = 0.55)	-0.10 (p = 0.44)

TWA = time weighted average; mG = milliGauss; RCM = rate of change metric; RCMS = standardized rate of change metric.

[a] Pearson correlation provided similar results after log-transforming the magnetic field exposure metrics to satisfy assumptions of normality.

Table M.2. Spearman's correlation coefficients and p-values for Abeta variables versus magnetic field exposure metrics for the two hours prior to the subjects' blood draw.[a]

Exposure metric	Abeta(1-40)	Abeta(1-42)	Abeta Ratio Abeta(1-42)/ Abeta(1-40)
Arithmetic TWA (mG)	-0.02 (p = 0.88)	-0.03 (p = 0.82)	-0.07 (p = 0.58)
Geometric TWA (mG)	0.12 (p = 0.36)	0.12 (p = 0.37)	0.08 (p = 0.52)
Cumulative Exposure (mG-hrs.)	-0.02 (p = 0.91)	-0.03 (p = 0.84)	-0.07 (p = 0.59)
Cumulative Exposure > 2 mG (mG-hrs.)	-0.04 (p = 0.78)	-0.10 (p = 0.44)	-0.16 (p = 0.23)
RCM (mG/15 seconds)	-0.06 (p = 0.63)	-0.14 (p = 0.28)	-0.20 (p = 0.12)
RCMS (per 15 seconds)	0.11 (p = 0.39)	0.06 (p = 0.65)	0.03 (p = 0.81)

TWA = time weighted average; mG = milliGauss; RCM = rate of change metric; RCMS = standardized rate of change metric.

[a] Pearson correlation provided similar results after log-transforming the magnetic field exposure metrics to satisfy assumptions of normality.

Table M.3. Spearman's correlation coefficients and p-values for Abeta variables versus work magnetic field exposure metrics over three days.[a]

Exposure metric	Abeta(1-40)	Abeta(1-42)	Abeta Ratio Abeta(1-42)/ Abeta(1-40)
Arithmetic TWA (mG)	0.03 (p = 0.82)	-0.02 (p = 0.88)	0.08 (p = 0.55)
Geometric TWA (mG)	0.15 (p = 0.25)	0.12 (p = 0.38)	0.05 (p = 0.71)
Cumulative Exposure (mG-hrs.)	0.05 (p = 0.71)	-0.02 (p = 0.87)	-0.09 (p = 0.51)
Cumulative Exposure > 2 mG (mG-hrs.)	0.00 (p = 0.99)	-0.07 (p = 0.60)	-0.13 (p = 0.31)
RCM (mG/15 seconds)	-0.08 (p = 0.55)	-0.16 (p = 0.23)	-0.21 (p = 0.11)
RCMS (per 15 seconds)	-0.05 (p = 0.71)	-0.07 (p = 0.57)	-0.04 (p = 0.76)

TWA = time weighted average; mG = milliGauss; RCM = rate of change metric; RCMS = standardized rate of change metric.

[a] Only two days of work magnetic field exposure for one subject. Pearson correlation provided similar results after log-transforming the magnetic field exposure metrics to satisfy assumptions of normality.

Appendix N

Magnetic field variability and Abeta

An adjusted analysis of variance model was used to assess the association between Abeta and measures of magnetic field variability. The models were adjusted for age, income, vegetable consumption, and physical work. Table N presents the adjusted least-squares means of the Abeta parameters by tertile of RCM and tertile of standard deviation of the magnetic field mean. Figures N.1 and N.2 graphically present the results for the Abeta ratio and the two measures of magnetic field variability.

Table N. Adjusted least-squares means of the Abeta parameters by tertile of RCM and standard deviation of the magnetic field mean.[a]

	Rate of Change Metric (RCM)			
Abeta parameter	1st Tertile	2nd Tertile	3rd Tertile	p-value[b]
Abeta(1-40)	156.0	175.8	148.6	0.547
Abeta(1-42)	293.8	307.1	182.3	0.050
Abeta Ratio (42/40)	1.91	1.72	1.08	0.010
	Standard Deviation of Mean Magnetic Field Exposure			
Abeta parameter	1st Tertile	2nd Tertile	3rd Tertile	p-value[b]
Abeta(1-40)	162.6	165.4	151.8	0.414
Abeta(1-42)	322.6	237.8	212.3	0.078
Abeta Ratio (42/40)	2.02	1.39	1.23	0.022

[a] Adjusted for age, income, vegetable consumption, and physical work.
[b] p-value testing for the difference between the means of the 3rd and 1st tertiles.

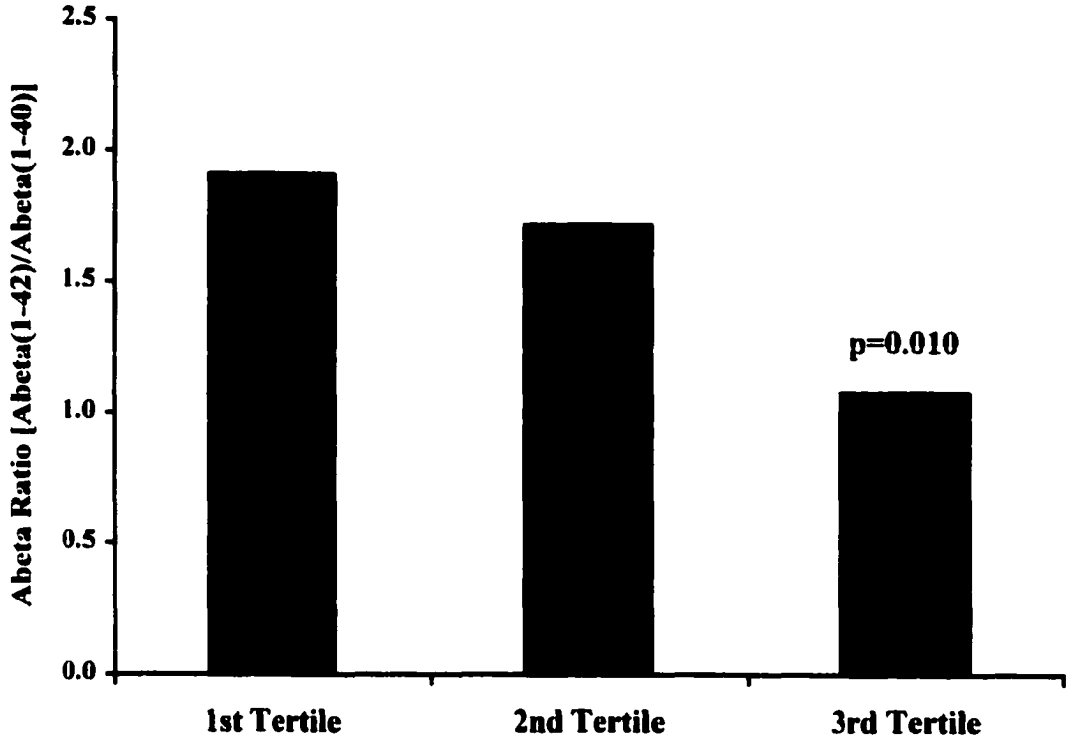

Figure N.1. Abeta Ratio [Abeta(1-42)/Abeta(1-40)] least-squares means by tertile of RCM, n = 60.[a]

[a] Means are adjusted for age, income, physical work, and vegetable consumption. p-value testing for the difference between the means of the 3rd and 1st tertiles. RCM = rate of change metric.

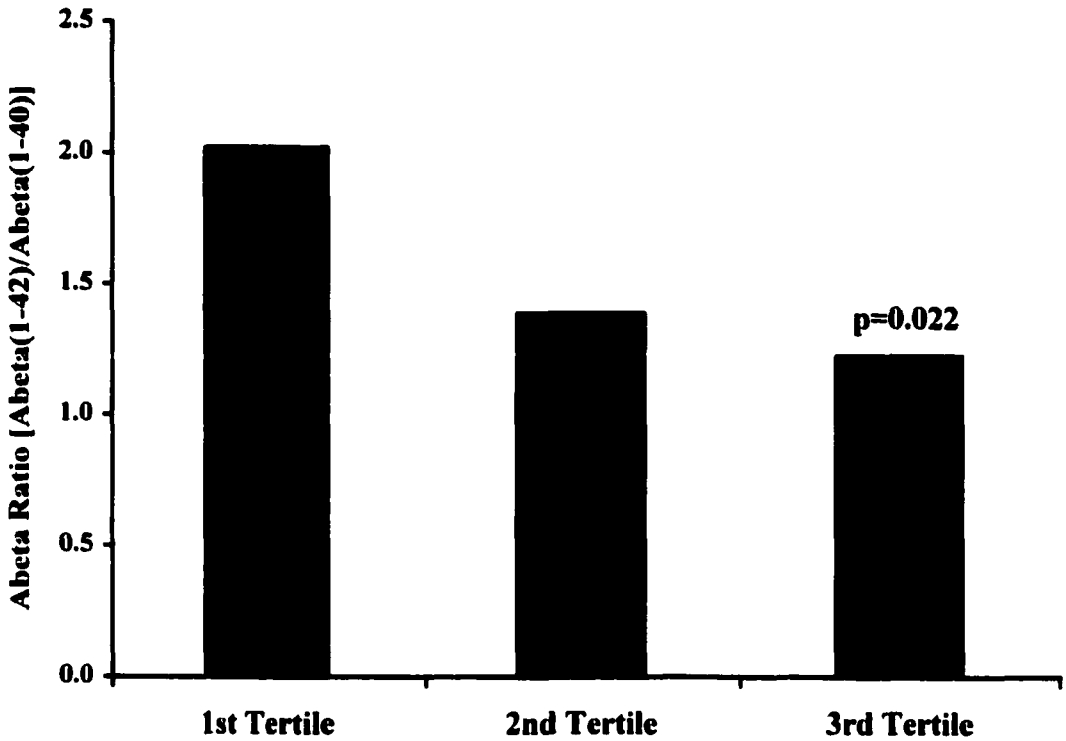

Figure N.2. Abeta Ratio [Abeta(1-42)/Abeta(1-40)] least-squares means by tertile of standard deviation of mean workplace magnetic field exposure, n=60.[a]

[a] Means are adjusted for age, income, physical work, and vegetable consumption. p-value testing for difference between the means of the 3rd and 1st tertiles.

Appendix O

Summary of 6-OHMS values for study population

Table O. Mean 6-OHMS values.[a]

6-OHMS variable	Distribution/ Generation workers (n = 44)	Administration/ maintenance workers (n = 16)	Total (n = 60)
Total overnight 6-OHMS excretion (ng)	11810	10534	11473
Overnight 6-OHMS Concentration (ng/ml)	27.7	26.3	27.3
Creatinine-adjusted overnight 6-OHMS concentration (ng/mg creatinine)	22.0	23.9	22.5
Post-work shift 6-OHMS Concentration (ng/ml)	4.4	2.3	3.8
Creatinine-adjusted post-workshift 6-OHMS concentration (ng/mg creatinine)	4.5	2.6	4.0

6-OHMS = 6-hydroxymelatonin sulfate

[a] Overnight 6-OHMS values refer to urine samples collected on the morning of the day of the blood draw. Missing overnight 6-OHMS values for two individual. Post-workshift values refer to urine samples collected on the afternoon of the blood draw.

Appendix P

Analysis of association between magnetic field metrics and post-workshift creatinine-adjusted 6-OHMS (6-OHMS/cr).

The association between individuals' work magnetic field exposure and post-workshift 6-OHMS/cr concentrations on the day of the blood draw was assessed using both analysis of variance (ANOVA) with the metric grouped by tertile and regression using the metric as a continuous variable. The results of both models, adjusted for age, month, and ambient light exposure, are presented in Table P. Using ANOVA there is no indication of a difference between the lowest and highest tertile of each metric, and there is no linear relationship with 6-OHMS/cr by tertile of exposure. Based on these analyses, adjusting for *a priori* confounders, there is no association between work magnetic field metrics and post-workshift 6-OHMS/cr on this day of observation.

Table P. Association between magnetic field exposure and post-workshift 6-OHMS/cr on the day of the blood draw.

Analysis of Variance[a]				Regression[a]
Magnetic Field Metric	p-value for Low versus High	Tertile of metric	Adjusted least-squares mean 6-OHMS/cr	p-value for metric
Mean	0.498	Low	2.70	
		Medium	1.62	0.638
		High	2.18	
Geometric mean	0.170	Low	3.10	
		Medium	1.63	0.465
		High	1.99	
CUME	0.829	Low	2.44	
		Medium	1.70	0.635
		High	2.25	
CUME > 2 mG	0.842	Low	2.32	
		Medium	1.86	0.639
		High	2.18	
RCM	0.888	Low	2.53	
		Medium	1.49	0.792
		High	2.44	
RCMS	0.552	Low	1.54	
		Medium	3.22	0.297
		High	1.86	

RCM = rate of change metric; RCMS = standardized rate of change metric; CUME = cumulative exposure; mG = milliGauss; 6-OHMS/cr = creatinine-adjusted 6-hydroxymelatonin sulfate.

[a] Both models are adjusted for age, month, and ambient light exposure.

Appendix Q

Correlation between 6-OHMS and Abeta

Table Q. Spearman's correlation coefficients (and p-values) for Abeta variables versus 6-OHMS variables.[a]

6-OHMS variable	Abeta(1-40)	Abeta(1-42)	Abeta Ratio Abeta(1-42)/ Abeta(1-40)
Total overnight 6-OHMS excretion (ng)	0.03 (p = 0.82)	0.07 (p = 0.62)	0.06 (p = 0.64)
Overnight 6-OHMS concentration (ng/ml)	0.03 (p = 0.84)	0.03 (p = 0.84)	-0.01 (p = 0.97)
Creatinine-adjusted overnight 6-OHMS concentration (ng/mg creatinine)	-0.08 (p = 0.56)	-0.04 (p = 0.76)	-0.02 (p = 0.88)
Post-work shift 6-OHMS concentration (ng/ml)	-0.13 (p = 0.33)	-0.17 (p = 0.20)	-0.16 (p = 0.22)
Creatinine-adjusted post-workshift 6-OHMS concentration (ng/mg creatinine)[b]	-0.16 (p = 0.22)	-0.22 (p = 0.08)	-0.21 (p = 0.11)

6-OHMS = 6 hydroxymelatonin sulfate

[a] Overnight 6-OHMS values refer to urine samples collected on the morning of the day of the blood draw. Missing overnight 6-OHMS values for two individual. Post-workshift values refer to urine samples collected on the afternoon of the blood draw.

[b] Pearson's correlation coefficients for this variable (using log-transformed values for post-workshift 6-OHMS/cr to achieve normality) were –0.12 (p = 0.37), -0.16 (p = 0.21), and –0.18 (p = 0.17) for Abeta(1-40), Abeta(1-42), and the Abeta ratio, respectively.

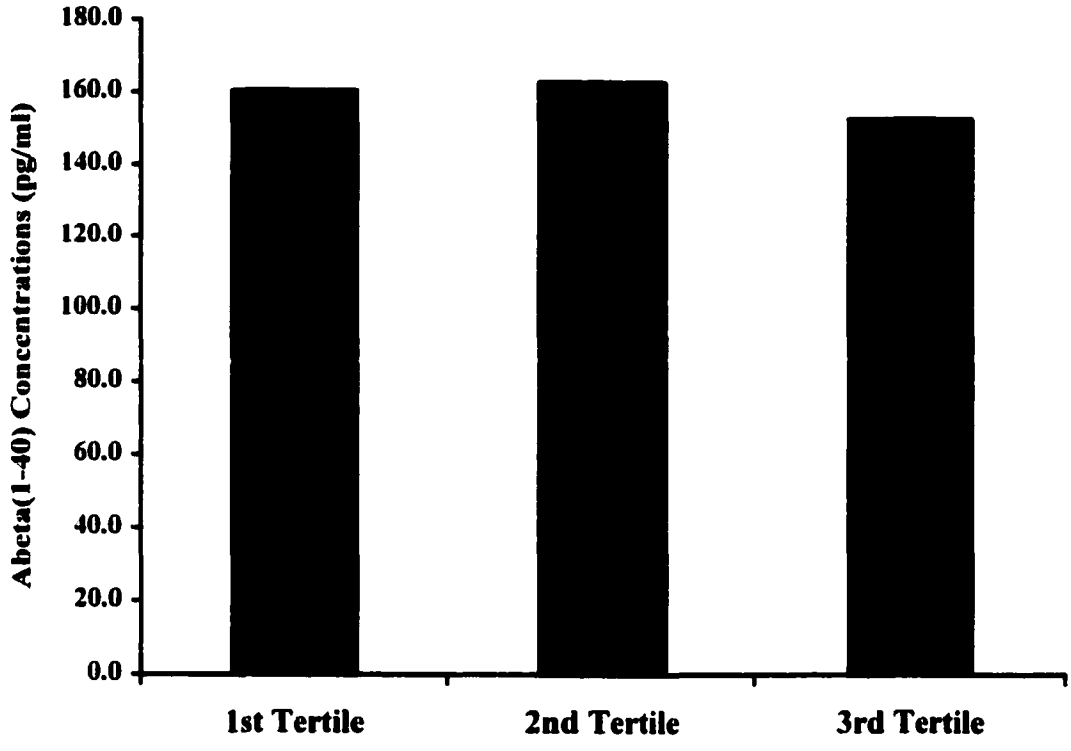

Figure Q.1. Abeta(1-40) Least-squares Means by Tertile of Creatinine-adjusted Post-workshift 6-OHMS, n = 60.[a]

[a] Means are adjusted for age, income, physical work, and vegetable consumption. p-value is for comparison of 3rd tertile versus 1st tertile.

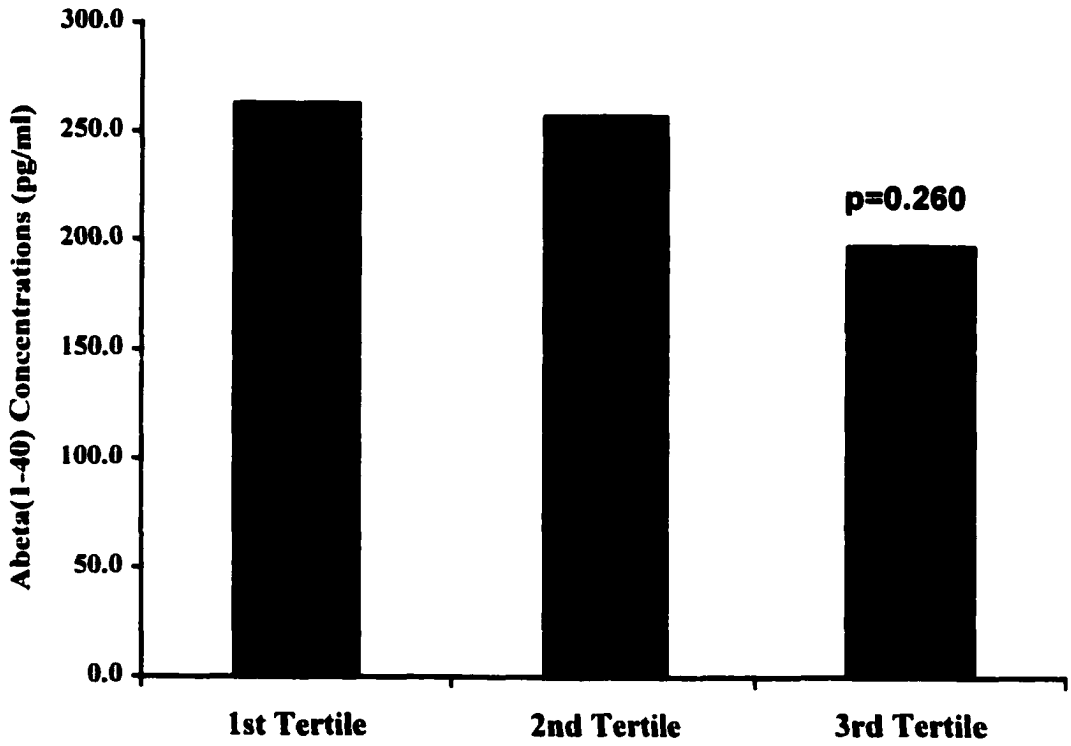

Figure Q.2. Abeta(1-42) Least-squares Means by Tertile of Creatinine-adjusted Post-workshift 6-OHMS, n = 60.[a]

[a] Means are adjusted for age, income, physical work, and vegetable consumption. p-value is for comparison of 3rd tertile versus 1st tertile.

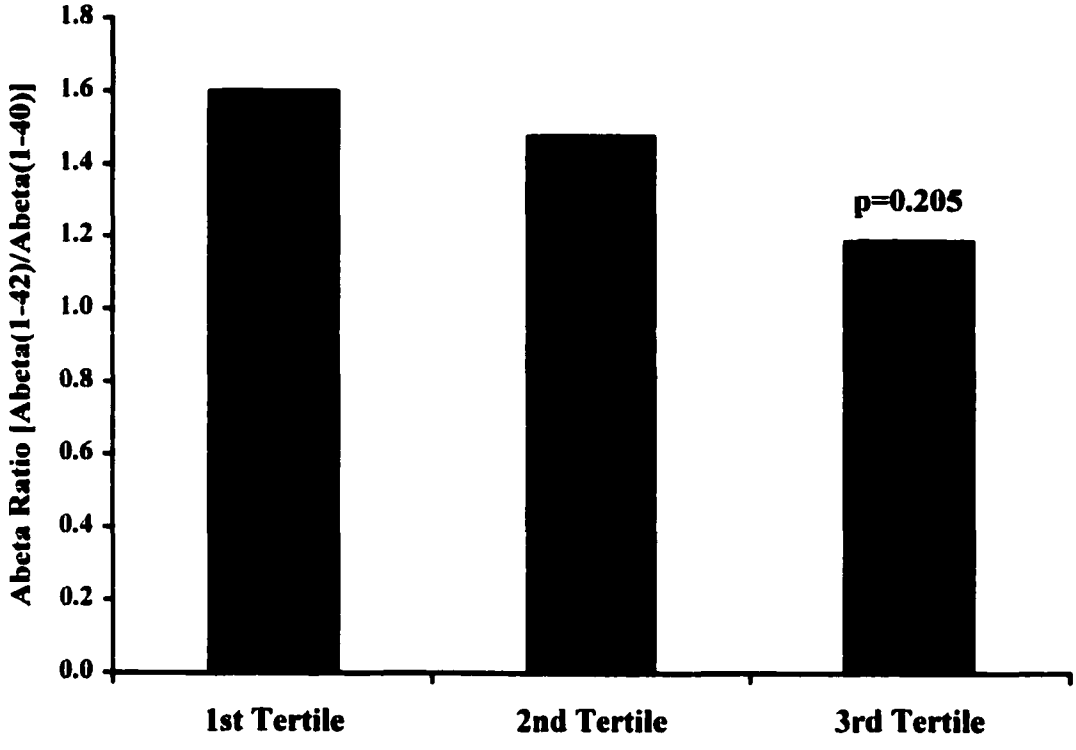

Figure Q.3. Abeta Ratio [Abeta(1-42)/Abeta(1-40)] Least-squares Means by Tertile of Creatinine-adjusted Post-workshift 6-OHMS, n = 60.[a]

[a] Means are adjusted for age, income, physical work, and vegetable consumption. p-value is for comparison of 3rd tertile versus 1st tertile.