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Influences of Physical Activity on Risk of Parkinson's Disease and Cognitive Decline in Elderly Hispanics

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Influences of Physical Activity on Risk of Parkinson's Disease and Cognitive Decline in Elderly Hispanics

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in Epidemiology

by

I-Fan Shih

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ABSTRACT OF THE DISSERTATION

Influences of Physical Activity on Risk of Parkinson's Disease and Cognitive Decline in Elderly Hispanics

by

I-Fan Shih

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2018

Professor Beate R. Ritz, Chair

The number of persons aged 65 years and older is expected to double to 92 million in the US by 2060, representing a potentially large social and financial burden of age-related neurodegenerative diseases such as Alzheimer's disease (AD) and dementias and Parkinson's disease (PD).Thus effective preventative public health strategies are essential. Regular physical activity (PA) and an active lifestyle have many health benefits, and evidence has accumulated that PA preserves brain structures and functions via multiple physiologic mechanisms, including mediation of inflammation and a general reduction of cardiovascular risk factors.

In the Parkinson's Environment and Gene (PEG) case-control population-based study, we enrolled 357 incident PD cases and 341 controls in central California and assessed PA levels via self-report of (1) overall PA over 4 age periods; (2) competitive sports; and (3) occupational histories. Our findings suggest that higher lifetime moderate to vigorous activity, especially

consistently high level of such activities throughout adulthood and sports activities in youth were negatively associated with PD risk, but we found no beneficial role for occupational physical activity.

We further examine how PA at the late-life influence the risk of dementia/cognitive impairment without dementia (CIND) using a prospective cohort, Sacramento Area Latino Study on Aging (SALSA) study, in which 1789 older Mexican Americans were enrolled at baseline and a majority actively followed between 1998 and 2008. We used Cox proportional hazards regression models to estimate the individual and joint effects of PA, apolipoprotein E (APOE) ε4 and diabetes status on risk of dementia/CIND and observed a nearly 10-fold increased risk among those with all three risk factors. Results from mediation analyses indicate that some of the PA effects on mortality and dementia/CIND but not depression might be mediated through well-known inflammatory pathways represented by biomarkers, specifically interleukins 6 (IL-6) and/or tumor necrosis factor - alpha (TNF-α). However, a large proportion of the PA effects on mortality, cognition, and mood seems to operate independently of the inflammatory pathways or the biomarkers we had available and thus remain unexplained.

Taken together, our results provide support for the hypothesis that PA protects against the onset of neurodegenerative diseases and all-cause mortality, and they suggest that anti-inflammatory action may partly explain the protective effects of PA on dementia/CIND and mortality.

The dissertation of I-Fan Shih is approved.

Mary N. Haan

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2018

DEDICATION

Dedicated to my inspiring parents, beloved family and to loving memory of my grandmother.

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Chapter 1. Background and Introduction

1.1 Parkinson's Disease

Epidemiology of Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD), characterized by progressive motor and non-motor impairments. Besides the cardinal motor symptoms, including resting tremor, bradykinesia, rigidity and postural instability, patients also suffer from sensory (anosmia, pain, paresthesia), autonomic (dysphagia, constipation, urinary incontinence) and neuropsychiatric (depression, apathy, dementia, hallucination) symptoms during the course of the disease.[1] All of these may limit patients' daily function and impose a substantial burden on the health-related quality of life of PD patients and their caregivers.[2]

The prevalence rates and incidence rates of PD increased smoothly with age, with prevalence rates increasing from 1-2% to 4-5% for persons over age 65 and over 85, respectively.[3] The age-standardized incidence rates for PD ranged between 14 and 19 per 100,000 person-years in different studies, while restricted to individuals aged 65 years and above, the incidence rate was 160 per 100,000 person-years.[4] The aging boomer population has led to ominous projections for PD that there may be 80 percent more Americans with the disease by 2030, leading to a large financial and caretaker burden.[5] The economic burden of Parkinson's disease is, at least, $14.4 billion in 2010 in the United States (approximately $22,800 per patient per year) and the economic burden is projected to grow substantially over the next few decades along with the disease prevalence.[6]

Neuropathology and lifestyle-related factors of Parkinson's disease

Although PD is a complex disease that involves many brain regions, the primary motor dysfunction of PD stem from the deterioration of the dopaminergic neurons in the pars compacta region of the substantia nigra, which leads to dopamine insufficiency in the striatum and later causes alterations in the activity of the neural circuits within the basal ganglia that regulate movement.[7] Dopamine is a neurotransmitter involved in cognition, motor activity, reward, mood, attention, and learning, and the Parkinsonism symptoms do not develop until 60-70% of dopamine neurons are gone and up to 80% of the dopamine content of the striatum is depleted.[4] As PD progresses and dopaminergic neurons die, neuronal loss with Lewy body formation also occurs in other brain regions. Lewy bodies are usually eosinophilic, intracytoplasmic inclusion bodies, containing alpha-synuclein, ubiquitin, and other proteins, which are another pathological feature of the disease.[7, 8] Increasing evidence indicates that inflammation, mitochondrial dysfunction, oxidative stress, the impairment of the ubiquitin-proteasome system and protein mishandling may be some of the molecular pathways resulting in PD.[7, 8]

The etiology of PD is not yet well understood but likely it involves both genetic and environmental factors.[4, 9] Over the past decade, lifestyle factors such as smoking and caffeine use have consistently been shown to be less common among those who later develop PD,[4, 9] but for these habits, reverse causation is a possible explanation due to the long prodromal period.[10] Less is known about other factors that may mitigate symptoms, particularly as it relates to 'healthy lifestyle' and especially physical activity (PA). After diagnosis, physical therapy, and exercise treatments are recommended to improve PD patients' daily function, motor impairments and

quality of life.[11-13] Since therapeutic exercise training is effective in reducing PD symptoms, it deserves our attention about the role lifetime physical activity may play for PD risk.

1.2 Cognitive Decline and Physical Activity in Elderly

As the world population ages, the number of persons aged 65 years and older is expected to double to 92 million in the US by 2060.[14] Cognitive change is common in older age and aging is a major risk factor for cognitive decline, Alzheimer's disease (AD) and other types of dementia. The prevalence of dementia in the US is approximately 14% in people over 71-year-old, with 10% attributed to AD.[15]

Over the past decade, many of the observational studies employed self-report instruments of activity (e.g., physical activity such as walking, sports, swimming, bicycling, etc.) at baseline interview and prospectively follow participants to examine cognition changes or events of dementia. The literature generally indicates that physical activity is positively associated with brain function throughout a lifetime, even in older ages.[16] A meta-analysis including 15 cohort prospective studies, with 30,331 non-demented participants, showed that being highly physically active at baseline had an overall 38% decreased the risk of cognitive decline compared with people having a sedentary lifestyle.[16] Several studies targeting older populations (ages 65 years and older) also showed similar results. In the Nurses' Health Study (n=18,766), active women, who walked ≥90 min/wk, had higher global cognitive scores than those less active (walking <40 min/wk) after 10-15 years follow up at ages 70-81 years.[17] Another population-based longitudinal study, Adult Change in Thought (ACT), followed 1,740 persons older than 65 years with intact cognition for

6.2 years and reported that people who exercised regularly (3 times or more per week) had a 32% reduced risk of dementia than those who did not.[18] Although longitudinal studies consistently supported the beneficial effects of exercise on brain function in elderly, mechanisms in humans are still to be better understood. Moreover, even though dementia is more prevalent among elderly of African-American and Hispanic ancestry than in non-Hispanics of the same age,[19] less is known whether levels of physical activity protect later cognitive decline in these minority populations.

1.3 Biological Mechanism: Physical Activity and Parkinson's Disease

There is growing evidence suggesting that physical exercise can trigger biochemical changes facilitating neuroplasticity as characterized by neurogenesis, synaptogenesis, angiogenesis and molecular adaptations, in the human nervous system.[20-22] Indeed, regular physical exercise decreases inflammation, induces antioxidant enzyme and defenses, and improves mitochondrial volume and function.[23, 24] The exercise-induced neuroprotective effects and its mechanisms are mostly shown in laboratory experiments with animal models. Studies of the effects of physical exercise on PD have mainly relied on the 1,2,3,6-tetrahydropyridine (MPTP)-lesioned mouse model and the 6-hydroxy-dopamine (6-OHDA)-lesioned rat model, using the two toxins to either bilaterally or unilaterally destroy nigrostriatal dopamine neurons and deplete dopamine.[25-27] Forced or voluntary exercise is introduced before, during, or immediately after toxin administration to study the exercise effects in combination with dopaminergic neurotoxins.[25-27] Although the cause or pathophysiology of these toxin models is different from PD in human, they still provide valuable insight into the possible neuroprotection and recovery effects of exercise.

Exercise-induced neuroprotective effects

Early studies have reported that animals exercised either before or soon after toxin was administration retained more dopamine neurons and dopaminergic terminals as well as had better motor performance than immobilized ones. Unilateral 6-OHDA-lesioned rats forced to use the impaired limb immediately after administrations had less behavioral deficit and dopamine metabolites sparing;[28] moderate treadmill training after toxin exposure was found to attenuate lesion-induced striatal dopamine and dopamine terminal markers, and improve behavioral deficits in terms of limb akinesia in 6-OHDA-lesioned rats or stride length during walking in MPTP-lesioned mouse;[29] exercise before toxin treatment also prevents the development of behavioral deficits, and loss of striatal dopamine and brain neurotrophic factors.[30] Forced or voluntary exercises stimulate neurotrophic factors that act on dopamine neurons in the striatum, reduce their vulnerability to neurotoxicants, and promote mitochondrial energy production and antioxidant defenses, which in turn may prevent behavioral and neurochemical deficits related to PD.[27, 30] Moreover, exercise induces expression of brain neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), and downregulation of the dopamine transporter (DAT) after exercise training.[31, 32]

Exercise-induced neurorestorative effects

Neuro-restorative effects in PD, on the other hand, are defined as the capacity of the remaining surviving dopaminergic neurons to functionally adapt after toxin-induced cell death used to model Parkinsonism.[33, 34] To investigate the neurorestorative effect, exercise training was provided several days after the toxin injection when cell death was considered complete.[35] Despite

90% striatal dopamine loss, MPTP-lesioned mice forced to exercise showed significantly improved motor impairment in terms of duration and velocity of running compared to non-exercising lesioned mice.[35] Moreover, exercise resulted in a down-regulation of striatal DAT and increased dopamine D2 transcript expression and nerve terminal glutamate immune-labeling in the MPTP-lesioned exercising mice.[35] These changes suggest that intensive exercise may restore (repair) motor function in mice to some degree by altering glutamate-dopamine interactions and neurotransmission.[35, 36] In a follow-up study by Petzinger et al., motor improvements in the MPTP-lesioned mice were associated with localized dopamine released in the dorsolateral striatum but not with total striatal dopamine levels.[37] Thus, in mice, exercise increases post-lesion dopamine neurotransmission by stimulating the vesicular release of dopamine, improving synaptic occupancy, and reducing dopamine clearance through reduced DAT expression.[34]

Alternatively, it has been suggested that consistently high levels of plasma urate may provide another plausible mechanism for neuroprotection. In human studies, high-intensity exercise has been found to induce a lasting elevation in plasma urate, a natural antioxidant and potent scavenger of iron and free radicals and higher urate levels have previously been associated with lower PD risk and slower disease progression mostly in men.[38, 39]

1.4 Biological Mechanism: Physical Activity, Inflammation and Cognition

Physical activity and cognition

Exercise-induced effects on brain function, particularly on cognitive function may work at the systems, molecular and cellular level.[21, 40] At the systems level, physical activity may improve

cognitive function by increasing neuronal activation in brain areas that are responsible for attention, learning, and memory. Electrophysiologic and neuroimaging studies have found increased neuroelectric activity, brain volume, and blood flow in these brain areas coupled with the better memory and cognitive performances after aerobic exercise training or in highly fit persons. [40]At the molecular level, physical activity induces the production of neurotrophins (e.g. BDNF) and growth factors (e.g. IGF-1, VEGF) in the brain, which are contributing to neural survival, growth, and synaptic plasticity.[41] Animal studies found that both BDNF gene and protein expression in the hippocampus and other parts of the nervous system (such as those important for visual discrimination and object recognition) are increased after short or long term, voluntary or forced exercise protocols.[41] Similar upregulation of exercise-induced central BDNF levels was found in healthy humans as well.[41] Insulin-like growth factor-1 (IGF-1), crucial for exercise-induced angiogenesis in the hippocampus, was also positively elevated with better cognitive performance both in animal and human studies.[21] Vascular endothelial growth factor (VEGF) is another growth factor that works together with IGF-1 to induce growth of blood vessels.[42] These factors are induced by physical activity and may improve cognition through increased angiogenesis, neurogenesis, and synaptogenesis at the cellular level.[40, 42]

Physical activity and inflammation

While transient inflammation is a protective response that stimulates healing processes after injury and infection, it is hypothesized that failure to completely terminate the immune response within a given timeframe may cause a systemic state of low-grade chronic inflammation associated with aging and many age-related diseases.[43] The most prominent inflammatory markers including

cytokines interleukin (IL)-6, IL-1, and tumor necrosis factor - alpha (TNF-α); their soluble receptors IL-1 receptor antagonist, TNF-α receptors and soluble IL-6 receptor; the acute phase protein C-reactive protein (CRP); and total leukocyte count.[44] Depending on their function, cytokines can be pro-inflammatory (e.g., IL-6, IL-1β, TNF-α) or anti-inflammatory (e.g., IL-4, IL-10, IL-13).

Inflammation and pro-inflammatory cytokines impair insulin--IGF-1 transduction and BDNF signaling in neurons, in which low levels of IGF-1 and/or BDNF are associated with cognitive impairment.[24] Exercise might counteract this negative effect not only via improvement of IGF-1 and BDNF signaling level but also reducing circulating pro-inflammatory cytokines.[24] The anti-inflammatory effects produced by regular physical activity is related to increased release of anti-inflammatory cytokines by skeletal muscles and reduced visceral fat.[45] During exercise, the cellular and circulating level of IL-6 increase remarkably during skeletal muscle contraction but return to resting levels within a short duration.[45] The transient increase in IL-6 levels subsequently triggers the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonists and suppresses TNF-α levels to reduce inflammatory effects.[45] In contrast, sedentary and inactive lifestyles cause visceral fat accumulation, which is commonly known to increase pro-inflammatory cytokines and reduce anti-inflammatory cytokines.[45, 46] Animal study results reporting reduced brain IL-1β in a mouse model of AD and brain inflammation in the ischemic rat after exercise training both support exercised-induced anti-inflammation effects.[24] Therefore, regular exercise that results in a reduction of pro-inflammatory cytokines and systemic inflammation may be a potential mechanism for cognitive decline.

1.5 Dissertation Objectives

The aim of this dissertation is to investigate the influence of physical activity on risk of neurodegenerative diseases, including PD and dementia. In chapter 2, we examine the risk of PD associated with lifetime physical activity assessed via self-report of (1) overall PA over 4 age periods; (2) competitive sports; and (3) occupational histories. In chapter 3, we examined whether PA at late-life, *APOE* ε4 and diabetes status act together to influence the risk of dementia/CIND in elderly Mexican American. In the same cohort, we furthermore examined whether plasma inflammatory markers explain the associations between PA and mortality, dementia/CIND, or depression risk using mediation analyses (Chapter 4).

Chapter 2. Lifetime Occupational and Leisure Time Physical Activity and Risk of Parkinson's Disease

2.1 Abstract

Introduction: While regular exercise has been shown to alleviate the motor symptoms of Parkinson's disease (PD), it remains unclear whether a physically active lifestyle may prevent PD.

Methods: To examine physical activities across the lifespan and risk of PD, we relied on data from a population-based case-control study that enrolled 357 incident PD cases and 341 controls. We assessed physical activity levels via self-report of (1) overall physical activity (PA) over 4 age periods; (2) competitive sports; and (3) occupational histories.

Results: PD risks were lower comparing the overall PA highest quartile (moderate to vigorous activities ≥180 metabolic equivalent task-hours/week (MET-h/wk)) with the lowest quartile (<47.8 MET-h/wk) in age-period 18-24 years (adjusted odds ratio (OR) 0.64, 95% confidence interval (CI) 0.40-1.02), and 45-64 years (OR 0.50, 95%CI 0.31-0.83) but not in age-period 25-44. Individuals who consistently engaged in overall PA at high levels (before age 65 years) had a 51% lower PD risk than those with low levels. Also, having participated in competitive sports prior to age 25 was inversely associated with PD (OR 0.53, 95% CI 0.31-0.91 for high level versus never). There was no association for measures of occupational physical activity though.

Conclusion: The long prodromal stage of PD makes it difficult to conclude whether insidious disease leads to a reduction of physical activity years before motor symptom onset and PD diagnosis. However, sports activities and high levels of overall PA in youth appear protective unless they are markers for biologic or genetic factors that lower PD risk.

2.2 Introduction

Parkinson's disease (PD) is characterized by progressive motor and non-motor impairments leading to disability, a considerable decline in health-related quality of life, and a large financial and caretaker burden in aging societies. Many clinical trials have shown beneficial effects of exercise therapies in people with PD as summarized in meta-analyses; for example, two recent meta-analyses found that aerobic exercise or physical therapy improved motor scores based on the Unified Parkinson's disease rating scale (UPDRS), as well as balance and gait compared with no intervention [13, 47]. Even though there is some evidence from animal experiments and PD intervention studies that intensive physical exercise induces neuroplasticity in the nigrostriatal dopaminergic system [25, 35, 36, 48], less is known about the role lifetime physical activity may play for PD risk.

Some cohort studies (Supplementary Table 1) have suggested that those physically inactive or reporting prolonged daily TV viewing are more likely to develop PD [49-54]. However, many of these cohort studies relied on self-report of physical activity levels or recorded activity patterns at baseline only [53, 54], accrued small numbers of incident PD cases or followed populations with a lifestyle different from the general population [52, 53]. In addition, most previous studies focused on leisure time activities while only two considered work-related activities [50, 52]. Activities at work and during leisure time likely entail very different physical demands, in terms of intensity, frequency and duration, and are known to affect all-cause mortality and cardiovascular disease differently [55, 56]. Our aim here is to examine whether the type and/or the timing of physical activity throughout adulthood affects the risk of PD.

2.3 Methods

Data collection and procedures described in this study have been approved by the University

of California, Los Angeles (UCLA), Institutional Review Board for human subjects, and written informed consent has been obtained from all participants.

Study Population

We conducted a population-based case-control study in largely agricultural counties in Central California. Details of the study have been provided elsewhere [57]. Briefly, we enrolled recently diagnosed PD patients (within 3 years of diagnosis) between 1998-2007, residing in Fresno, Kern, or Tulare counties, who at recruitment had lived in California for 5 years or more. Potential cases and controls were contacted by mail, by telephone, or both with eligibility criteria including: (1) being at least 35 years of age; (2) not too ill to participate; (3) currently living in one of the three designated counties; (4) having lived in California for five years or more; and (5) having PD for cases.

Of the 1,167 PD patients who responded to invitations, we excluded 604 who had their initial PD diagnosis 3 years prior to contact or did not fulfill the above inclusion criteria. Of the 563 eligible cases, 90 were too ill to be examined, moved or died prior to the exam. The remaining eligible cases were examined by movement disorder specialists from UCLA to confirm PD diagnoses while 94 did not meet published criteria for idiopathic PD [58], an additional 13 were reclassified as not having idiopathic PD during our follow-up study [59], and 6 withdrew between examination and interview. Of the remaining 360 cases, 357 provided complete information on physical activity.

Population controls 65 years or older were first identified from Medicare lists (in 2001). Later, due to the implementation of the Health Insurance Portability and Accountability Act (HIPPA), we recruited around 70% of controls of all ages from residential parcel tax assessor records in the tri-county area. Of the 1,212 potential controls, 457 were ineligible, and 409 declined participation

due to illness or moving; a total of 341 individuals provided complete information on physical activity.

Assessment of Physical Activity

Trained interviewers blinded to case/control status conducted structured telephone interviews to obtain demographic and physical activity information, including self-report of (1) overall physical activity level across four age periods; (2) history of participation in competitive sports; and (3) occupational histories to create a job exposure matrix (JEM) and estimate occupational physical activity.

Overall Physical activity

Participants were asked to report an average number of days per week and the average number of hours per day during which they performed mild, moderate, or vigorous physical activity at work and leisure time, during 4 periods of adulthood: 18-24, 25-44, 45-64, and ≥ 65 years. Definitions and examples for the intensity of activities were provided during the interview. To account for both the effects of duration and intensity, we assigned metabolic-equivalent (MET) values to the activity intensities (vigorous activities as 8 and moderate activities as 4) [60] and created a cumulative physical activity measure — MET-hour per week (MET-h/wk) at each age period. Previous studies suggested that only moderate to vigorous activities were associated with PD risk and no effect was observed for mild activities [49, 50]; therefore we set "mild" activities to a MET value of zero to maximize the specificity of the physical activity measures. We also calculated the sum of the MET-hour per week for every year of adulthood before the index date (PD onset in cases, interview date in controls) and divided by the total number of adult years to derive the average lifetime activity score.

Competitive sports history

If a participant reported ever having engaged in competitive sports, we collected information about the type of sports, and ages at which they started and stopped. For every type of sport, a MET value was assigned according to published standard equivalents [61]. We then multiplied the MET value with the reported years for each sport and summed those to derive cumulative sports measures (MET-year).

Occupational history

Participants were asked to report job titles, tasks, companies, industries, and duration (years) and frequency (hours per week) for all jobs in which they had worked for 6-months or more throughout their lifetime. We created a JEM to estimate occupational physical activity by coding information about jobs and industries based on the Integrated Public Use Microdata Series (IPUMS-USA) 2000 Occupation Code System [62], and assigned MET value to each job code [63]. We first multiplied the MET value with the reported years in each job, and summed those to derive cumulative occupational physical activity measures, and then calculated an average lifetime score by dividing the cumulative score by the total number of working years.

Statistical analyses

Logistic regression analyses were performed using SAS software version 9.3 (SAS Institute, Inc., Cary, North Carolina), with adjustment for age (continuous), gender, race (white, non-white), education (<12 years, 12 years, >12 years), smoking status (never, past, or current smoker), having a 1st degree family member with PD (yes, no), residential pesticide exposures (ever or never exposed) [64] and pesticide exposure estimates previously derived from a JEM (never, low, median or high exposure) [65]. We reported odds ratio (OR), 95% confidence intervals (95%CI), and p-values for trend based on the median of each exposure category.

We categorized the physical activity scores into quartiles based on the distribution of average

lifetime MET scores in controls: <47.8, 47.8-93.0, 93.0-180.0, ≥180.0 MET-h/wk. Participants who never performed moderate and/or vigorous physical activities and those who fell in the 1st quartile of the MET distribution were considered less active and formed our reference group. Furthermore, we examined whether changes in overall physical activity over the lifetime until age 64 were associated with PD risk. For each age period, participants were categorized as having high or low activity based on the overall median (93.0 MET-h/wk). We compared those who reported a consistently high activity, and those who reported either low-high or high-low trajectories to those who reported consistently low activity throughout life. For occupational physical activity measures, we categorized cumulative (MET-year) and average (MET) scores into quartiles based on the control distribution in each age period, and for cumulative sport activity scores, we considered those who never participated in any strenuous sport as the reference and examined age periods specific tertiles (MET-year). We also included all three measures in the same model to mutually adjust for the different types of activity.

In sensitivity analyses, we stratified by gender or excluded subjects with a PD diagnosis prior to age 60. Examining occupational physical activity, we also excluded participants with high occupational pesticide exposures, because we previously found pesticide exposure to contribute to PD risk in this population [65]. To assess the potential influence of preclinical changes in physical activity due to insidious disease onset, we used exposure lagging and discounted activities within 10-years or 20-years prior to the index date for cumulative measures.

2.4 Results

Study participants were in average 68 years of age at eligibility screen and predominantly white (Table 1). Cases were more frequently male, less highly educated, never smokers, and residentially and occupationally more heavily exposed to pesticides. The mean score on the Hoehn

and Yahr scale was 2.10 (range 1-5) at baseline (mean PD duration: 1.71±1.18 years).

Higher levels of self-reported lifetime physical activity were inversely associated with PD risks (Table 2). Specifically, we found a 44% (OR 0.56, 95% CI=0.34-0.92) lower risk of PD among those with moderate to the vigorous physical activity of at least 180 MET-hours per week on average in adulthood compared with the less active reference group (<47.8 MET-h/wk). Similar results were obtained for all age periods except for activity during 25-44 years of age. Examining the influence of changes in physical activity from youth to later adulthood, those who remained highly active throughout their lifetime were at the lowest risk of PD (OR=0.49, 95%CI 0.32-0.76), followed by those with a high-low or a low-high trajectory, compared with those who were consistently less active (Fig. 1).

Having participated in competitive sports was inversely associated with PD risk (Table 3, Supplementary Table 2). Compared with individuals who never participated in competitive sports, for those who reported a high sports activity level prior to 25 years of age, we estimated a 47% lower risk of PD (OR 0.53, 95%CI 0.31-0.91; p for trend = 0.04). Cumulative or average scores for occupational physical activity were not associated with PD risk (Table 3).

In sensitivity analyses, no apparent gender-specific effect was found for overall physical activity or competitive sports measures, and higher levels of occupational physical activity did not reduce PD risk overall or in males only (Supplementary Table 3). Results from lagged analyses that excluded physical activity 10-years or 20-years prior to PD diagnosis (Supplementary Table 4) for cumulative measures were similar to unlagged estimates. Excluding participants with high occupational pesticide exposure (Supplementary Table 5) or excluding PD cases diagnosed prior to age 60 also did not substantially change all physical activity estimates (results not shown).

2.5 Discussion

In this population-based case-control study, we examined three different measures of physical activity and found that higher lifetime moderate to vigorous activity, especially consistently high level of such activities throughout adulthood, reduced the risk of developing PD. Controls who did not develop PD had more often engaged in strenuous competitive sports in their youth. However, occupational physical activity did not influence PD risk.

Our findings agree with a meta-analysis of five prospective studies reporting that being physically active reduces PD risk with a 34% lower risk estimated for the highest versus the lowest activity levels [52]. Some of these studies, however, only examined leisure-time physical activity at baseline (between age 50-60) [49, 51, 66], and did not account for other types of physical activity (e.g. occupational, household and commuting activities). A Swedish study [52], reported an inverse association with PD risk for the sum of household, commuting and leisure time exercise and total physical activity, but no associations for occupational activity or leisure time exercise when analyzed separately. Similarly, our overall physical activity measure included activities at work and leisure time, and higher activities according to this overall measure were inversely associated with PD risk. Our occupational measure covered all lifetime job-related activities and considered intensity and duration while the Swedish study only asked about job intensity in the past year [52]. Both studies do not support a protective role for occupational activities. Interestingly, the lack of beneficial effects for job-related physical activity in cardiovascular disease and all-cause mortality [55, 56] has recently been coined the 'health paradox' of physical activity [67].

We collected physical activity data according to age periods and found inverse associations with PD in all except the 25-44 year period. Careers and child rearing demands may restrict leisure time or sports activities in this period such that occupational activity may be the main activity to

overall physical activity. The only other paper [50] that examined physical activity during several age periods reported only on leisure time activity and reported inverse associations with PD during 35-39 years and 50-60 years of age but not in young adulthood (ages 15-29). Yet, our evaluation of activity trajectories throughout adulthood is consistent with this study's findings suggesting that individuals who remain highly active throughout life are at lowest risk of PD [50].

Similar to previous studies, we cannot dismiss the possibility of reverse causation because decreasing physical activity before disease onset might be a prodromal PD symptom. However, our estimates did not substantially change after excluding physical activity reported during 10- or 20-years before diagnosis. We also found that those who were consistently active throughout life or active in at least one of the age periods were at lower risk of PD, compared with those maintaining low physical activity levels throughout life. This suggests that staying or becoming active in adulthood may protect against PD. Further, the protective associations found for high physical activity and sports activities in youth would not be affected by reverse causation unless an active lifestyle depends on genetic factors that also lower PD risk.

Physical activity may reduce the risk of PD by increasing cerebrovascular circulation and improving the production of neurotransmitters, including neurotrophic substances [25, 36]. In rodent models of PD, animals forced to exercise before or after toxin treatments had more remaining dopaminergic neurons and terminals as well as less motor deficits than immobilized animals [29, 30]. Forced exercise was found to stimulate neurotrophic factors and downregulation of the dopamine transporter, which may contribute to neuroplasticity and reduce vulnerability to neurotoxicants [25, 36]. In early stages of PD, 8 weeks of high-intensity treadmill training (MET ≥3 or 60-80% age-adjusted maximal heart rate) has been shown to induce cortico-motor excitability and an increase in dopamine D2 receptor binding potential in the dorsal striatum, along with motor function

improvement [35, 48]. Alternatively, high-intensity exercise may induce elevations in plasma urate [38], an anti-oxidant, and high urate levels have been associated with lower PD risk and slower disease progression [39].

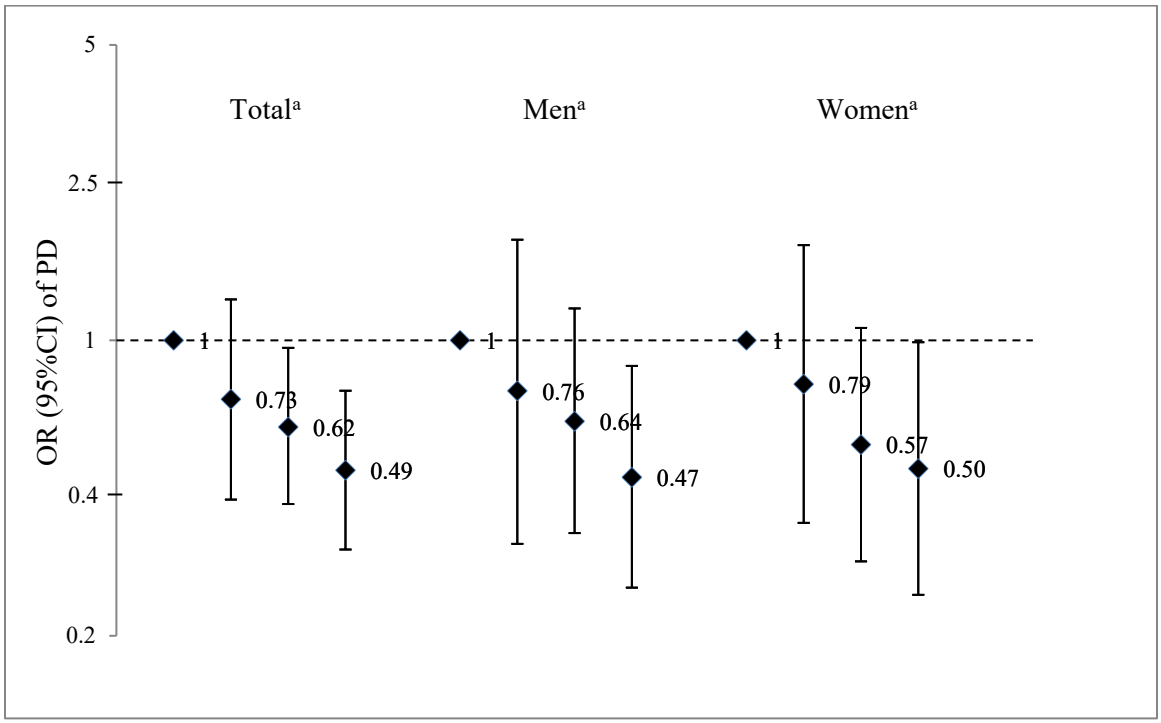
Compared with other studies, we measured physical activity levels more comprehensively using multiple approaches. First, we accounted for the two most important sources of physical activity – occupation and leisure time sports activity – by age periods. This allowed us to evaluate the effect of changes in physical activity levels, reflecting a life course perspective and possibly identify periods of importance for interventions. Second, to overcome some of the recall problems in our case-control design, we asked participants to report their lifetime history of competitive sports and job-related activities. Based on the latter, we created an exposure matrix for which raters assigned physical activity levels to job titles and tasks while blinded to case status. To our knowledge, we are the first PD study that used a JEM to examine occupational activity. Though the possibility exists that cases spent more effort to recall details of their job histories, we found that the reported number of occupations and work years was similar for cases and controls [65].

Our study with nearly 360 PD cases enrolled a substantially larger number of PD affected participants than most previous studies [52, 53, 66, 68]. Moreover, ours is the only epidemiologic population-based study in which movement disorder specialists examined 71% of patients multiple times over almost a decade to confirm a diagnosis of idiopathic PD, minimizing disease misclassification potential. PD ascertainment in prospective cohort studies is generally based on self-reported PD diagnoses and medical records review that relies heavily on a patients' access to quality health care [49, 50, 66, 68]. PD diagnoses strongly depend on clinical evaluations, thus diagnostic and reporting errors may be differential for those who are more health conscious and thus more physically active.

The present study has several limitations. Our JEM approach assumes that everyone with the same job title/tasks had a similar level of physical activity and thus was given the same MET value. This inevitably introduces non-differential misclassification errors because of likely within-job variability. Moreover, female participants may simply report housewife/homemaker, and this category is hard to judge in terms of physical activity. In our farming population, 'housework' may also be physically intensive for women who engaged in farming or gardening but do not consider this a job – these activities, however, would have been captured by our overall physical activity measure. Furthermore, our study was conducted in largely agricultural counties in Central California, which may limit the generalizability of our findings. Given the high correlation between active manual labor on farms and occupational pesticide exposure, controlling for pesticide exposure was important. Also, sensitivity analyses excluding participants with high occupational pesticide exposure did not change our results for occupational physical activity. An advantage of our rural study population is that our study included a wide range of occupational PA including physically demanding farm labor that should have increased our ability to detect associations. Although non-differential exposure misclassification remains a concern and may have biased our results towards the null, our findings indicate that the beneficial effects of overall physical activity and leisure time sports activity are not observed for occupational physical activity in this agricultural population.

In conclusion, we found that lifetime overall physical activity, and participation in competitive sports during young ages were negatively associated with PD risk, but found no beneficial role for occupational physical activity. Our results provide further support for a previous meta-analysis of 5 studies that concluded higher leisure-time physical activity levels are associated with lower PD risk [52].

2.6 Figure and Tables



[a] Left to right:

Low-Low trajectory (reference group): low activity at 18-24, 25-44 and 45-64 age periods.

Low-High trajectory: low activity at 18-24, and high activity at either 25-44 or 45-64 or both age periods.

High-Low trajectory: high activity at 18-24, and low activity at either 25-44 or 45-64 or both age periods.

High-High trajectory: high activity at 18-24, 25-44 and 45-64 age periods.

Figure 2-1. Multivariable-adjusted odds ratios (OR) with 95% confidence intervals (CI) of Parkinson's disease (PD) according to changes of overall moderate to vigorous physical activities before age 65.

The analysis adjusted for age, gender, race, education, smoking, family history of PD, and residential and occupational pesticide exposures.

Table 2-1. Sociodemographic characteristics of the study population in Central Valley of California, 2001-2007.

	PD Cases		Controls	
	N=357	%	N=341	%
Mean age (years) [SD]	68.29	±10.22	68.20	±11.42
Gender				
Male	205	57	176	52
Female	152	43	165	48
Race				
White	287	80	279	82
Non-white	70	20	62	18
Education				
< 12 years	66	18	38	11
12 years	96	27	64	19
> 12 years	195	55	239	70
Cigarette smoking status				
Never	187	52	146	43
Former	150	42	161	47
Current	20	6	34	10
PD Family History				
Yes	52	15	37	11
No	305	85	281	82
Residential pesticide exposure				
Dithiocarbamates				
Never	198	55	228	67
Ever	159	46	113	33
Organochlorines				
Never	148	41	159	47
Ever	209	59	182	53
Organophosphorus				
Never	60	17	83	24
Ever	297	83	258	76
Paraquat				
Never	128	36	139	41
Ever	229	64	202	59
Occupational Pesticide exposure				
None	227	64	242	71
Low	23	6	25	7
Medium	56	16	47	14
High	51	14	27	8

Table 2-2. Multivariable-adjusted odds ratios (aOR) with 95% confidence intervals (CI) and p-for-trend for Parkinson's disease (PD) risk by four age periods of self-reported overall moderate to vigorous physical activity[a].

Overall physical activity [MET-hour/week][b]	PD/Control	aOR	95%CI	P-for-trend[c]
Lifetime Average				
<47.8	107/84	1 (Reference)		
47.8-93.0	75/79	0.68	0.43-1.07	
93.0-180.0	73/79	0.68	0.43-1.07	
≥180.0	81/78	0.56	0.34-0.92	0.05
18-24 years				
<47.8	86/68	1 (Reference)		
47.8-93.0	82/69	1.00	0.62-1.62	
93.0-180.0	63/79	0.64	0.39-1.05	
≥180.0	105/104	0.64	0.40-1.02	0.03
25-44 years				
<47.8	111/93	1 (Reference)		
47.8-93.0	67/73	0.78	0.49-1.22	
93.0-180.0	53/62	0.73	0.45-1.19	
≥180.0	105/92	0.82	0.53-1.28	0.33
45-64 years				
<47.8	136/112	1 (Reference)		
47.8-93.0	65/67	0.82	0.52-1.29	
93.0-180.0	63/58	0.97	0.60-1.56	
≥180.0	65/72	0.50	0.31-0.83	0.01
≥65 years				
<47.8	145/112	1 (Reference)		
47.8-93.0	51/48	0.89	0.54-1.46	
93.0-180.0	24/35	0.50	0.27-0.92	
≥180.0	18/21	0.65	0.31-1.37	0.08

[a] Logistic regression models adjusted for age, gender, race, education, smoking, family history of PD, and residential and occupational pesticide exposures.

[b] Overall Physical Activity (MET-h/wk) = 8*vigorous activity hour/week + 4*moderate activity hour/week; quartiles according to the average lifetime physical activity distribution in controls.

[c] Linear trend was tested using the midpoint of each exposure category as a continuous variable in the regression model.

Table 2-3. Multivariable-adjusted odds ratios (aOR) with 95% confidence intervals (CI) and p-for-trend for Parkinson's disease (PD) risk by competitive sports and occupational activity[a].

Cumulative physical activity [MET-year][b]	PD/Control	aOR	95%CI	P-for-trend[c]
Competitive Sports				
Lifetime				
Never	184/160	1 (Reference)		
<42	69/61	0.99	0.63-1.56	
42-105	58/59	0.94	0.58-1.52	
≥105	40/58	0.62	0.37-1.04	0.06
18-24 years				
Never	192/168		1	
<40	63/59	0.97	0.61-1.52	
40-91	66/56	1.06	0.67-1.70	
>91	30/55	0.53	0.31-0.91	0.04
Occupational Activity				
Lifetime				
<56.7	89/85	1 (Reference)		
56.7-89.3	69/86	0.76	0.47-1.22	
89.3-130.8	86/84	0.85	0.51-1.43	
≥130.8	109/85	0.78	0.44-1.39	0.49
18-24 years				
<12.0	122/113	1 (Reference)		
12.0-22.5	70/76	0.92	0.60-1.43	
22.0-37.6	66/74	0.76	0.47-1.21	
≥37.6	94/76	0.97	0.59-1.59	0.86
25-44 years				
<30.7	90/102	1 (Reference)		
30.7-50.0	94/83	1.46	0.92-2.33	
50.0-65.8	62/75	0.87	0.52-1.46	
≥65.8	106/79	1.21	0.70-2.08	0.72
45-64 years				
<22.5	121/126	1 (Reference)		
22.5-36.5	71/68	0.97	0.61-1.55	
36.5-52.5	69/73	0.86	0.53-1.39	
≥52.5	91/72	0.86	0.52-1.42	0.51

[a] Logistic regression models were adjusted for age, gender, race, education, smoking, family history of PD, and residential and occupational pesticide exposures.

[b] Competitive Sports = sum of sport MET value*year of participation (MET-year); tertiles – age periods specific .

Occupational Activity = sum of job MET value*year of participation (MET-year); quartiles – age periods specific.

[c] Linear trend was tested using the midpoint of each exposure category as a continuous variable in the regression model.

2.7 Supplement

Supplementary Table 2-S1. 7 Cohort studies of lifetime physical activity and risk of developing Parkinson's disease

Author (year)	Cohort name, Country, Gender	Baseline/Age (year)/Follow-up (years)	Case N/ Cohort N	Activity measure	Case identification	Covariates Adjustment	Main results
Yang (2014)	National March Cohort, Sweden, Male and female	1997 / mean=50.3 / mean=12.6	286 / 43,368	Total PA: 24-h EE questionnaire, tertiles (MET-hours/day) Household and commuting: <2, 3-4, 5-6, >6 hours/week (h/wk) Leisure time exercise: tertiles (MET-hours/day) Occupational PA: sedentary, moving little, strenuous General PA: sum of household and commuting, leisure time and occupational, tertiles (MET-hours/day)	Swedish national patient register, primary and secondary PD diagnoses	Age, gender, education, smoking, alcohol, caffeine, BMI	Total PA medium vs. low: HR=0.55 (95%CI 0.35-0.87), male Household and commuting >6 vs. <2h/wk: HR=0.57 (95%CI 0.39-0.83), all; HR=0.50(95%CI 0.31-0.81), male Occupational PA strenuous vs. sedentary: HR=0.74(95%CI 0.45-1.22), all General PA high vs. low: HR=0.72 (95%CI 0.53-0.99), all; HR=0.53(95%CI 0.33-0.85), male
Sääksjärvi (2014)	Mobile Clinic Health Examination Survey, Finland, Male and female	1973-1976 / 50-79 / max=22	101 / 6,715	Leisure-time PA: none, light activity ≥4 h/wk, heavy activity≥3 h/wk	Nationwide register for prescription drug reimbursement	Age, gender, education, alcohol, caffeine, smoking, BMI, community density, occupation	Leisure-time PA heavy vs. none: RR=0.27(95%CI 0.08–0.90), all; RR=0.25(95%CI 0.06-1.09), excluded the first 10 years of follow-up*
Kyrozis (2013)	EPIC-Greece population-based cohort, Greek, Male and female	1993-1999 / 20-86 / mean=8.45	88 / 25,407	Self-reported both occupational and leisure-time PA, and calculated as daily MET-hours (continuous)	Self-report PD diagnosis or medication use with phone validation	Age, gender, marital status, education, farming, smoking, caffeine, BMI, energy intake	HR=0.95 (95%CI 0.90-1.00)
Xu (2010)	NIH-AARP (American Association of Retired	1996-1997 / 50-71 / max=10	767 / 213,701	Moderate to vigorous leisure time PA at ages 15-18, 19-29, 35-39 and in past 10 years: never, <1, 1-3, 4-7, >7 h/wk	Self-report with confirmed medical record	Age, gender, race, education,	Leisure time PA Moderate to vigorous activities >7 vs. <1h/wk: Ages 35-39: OR=0.62 (95%CI 0.48-0.81)

	Persons) Diet and Health Study cohort, United States, Male and female			Occupational PA: sitting, standing, walking, light or heavy lifting Sports at ages 15-18: <1, 1–2, 3–4, ≥5 times/wk		smoking, caffeine	Past 10y: OR=0.65; 95%CI 0.51–0.83) Occupational PA heavy lifting vs. sitting: OR= 0.63 (95%CI 0.39-1.02)
Thacker (2008)	Cancer Prevention Study II Nutrition Cohort, United States, Male and female	1992-1993 / mean=63 / max=10	413 / 14,325	Leisure-time PA: self-reported frequency/duration of light, moderate and vigorous activities (MET-h/wk) at baseline and at age 40.	Self-report with confirmed medical record	Age, education, caloric intake, dairy intake, smoking, alcohol, caffeine, BMI, pesticide, ibuprofen	Moderate to vigorous activities ≥16(M) or ≥11.5(F) MET- h/wk vs. none or light activities only at baseline: RR=0.6 (95%CI 0.4-1.0) Moderate to vigorous activity at age 40 was not associated with PD risk.
Logroscino (2006)	Harvard Alumni Health Study, United States, Male only	1988 / mean=67.6 / 10	101 / 10,714	Leisure time PA: self-reported frequency/duration; quartiles EE (<1000 to >3000 kcal/wk) Sports participation during college: <5, ≥5, unknown h/wk, varsity athlete	Self-report and death certificates	Age, smoking, tea, caffeine, history of CVD, cancer	EE >3000 vs. <1000 kcal/wk: RR=0.64 (95%CI 0.36-1.13); p for trend=0.12
Chen (2005)	Health Professionals Follow-Up Study (male) and Nurses' Health Study (female), United States	1986 / HPFS: 40-75; NHS: 30-55 / max=14	252 / 48,574 (M) 135 / 77,254 (F)	Leisure time PA: self-reported frequency/duration; quintile (MET-h/wk) Youth strenuous exercise: self-reported months per year spent ≥2 days/wk on strenuous exercises	Self-report with medical record confirmation	Age, smoking, energy, caffeine, lactose and alcohol intake, BMI	Leisure time PA highest vs. lowest quintile: RR=0.7 (0.5-1.1); vigorous only: RR=0.5 (0.3-0.9), male Youth strenuous exercise ≥10 vs. ≤2 months/year: 0.4 (95%CI 0.2-0.7), male; RR=0.5 (95%CI 0.2-1.4), female

PD, Parkinson's disease; PA, physical activity; MET, metabolic equivalent tasks; EE, energy expenditure; h/wk, hours per week; BMI, body mass index; CVD, cardiovascular disease; HR, hazard ratio; RR, relative risk; OR, odds ratio; CI, confidence interval.

* Less than 5 PD cases in the heavy group

Supplementary Table 2-S2. Distribution of competitive sports history (reported as ever participated) case status among study participants in Central Valley of California, 2001–2007

Competitive sports (MET)	All subjects (N=698)		PD cases (N=357)		Controls (N=341)	
	N	%	N	%	N	%
Basketball (7)	144	20.63	69	19.33	75	21.99
Baseball (5)	126	18.05	59	16.53	67	19.65
Football (8)	126	18.05	60	16.81	66	19.35
Track and field (7)	87	12.46	40	11.20	47	13.78
Softball (5)	58	8.31	26	7.28	32	9.38

a. We show only the top 5 most commonly reported competitive sports.

b. Note: Participants may have participated in more than one sports.

Supplementary Table 2-S3. Gender-stratified multivariable-adjusted odds ratios (aOR) with 95% confidence intervals (CI) and p-for-trend for Parkinson's disease (PD) risk by overall moderate to vigorous physical activity, competitive sports and occupational activity[a].

Physical activity[b]	Men				Women			
	PD/Control	aOR	95%CI	P-for-trend[c]	PD/Control	aOR	95%CI	P-for-trend[c]
Overall Physical Activity								
<47.8	43/30	1 (Reference)			64/54	1 (Reference)		
47.8-93.0	41/38	0.73	0.37-1.47		34/41	0.63	0.34-1.17	
93.0-180.0	48/42	0.74	0.38-1.45		25/37	0.56	0.28-1.12	
≥180.0	58/53	0.50	0.25-1.02	0.07	23/25	0.68	0.32-1.44	0.25
Competitive Sports								
Never	75/53	1 (Reference)			109/107	1 (Reference)		
<42	44/37	0.89	0.48-1.66		25/24	1.16	0.58-2.31	
42-105	47/39	0.98	0.53-1.82		11/20	0.70	0.29-1.71	
≥105	34/45	0.60	0.33-1.12	0.10	6/13	0.63	0.22-1.85	0.32
Occupational Activity								
< 56.7	12/11	1 (Reference)			77/74	1 (Reference)		
56.7-89.3	29/35	0.86	0.31-2.39		40/51	0.81	0.46-1.45	
89.3-130.8	63/61	1.17	0.44-3.13		23/23	1.02	0.48-2.16	
≥130.8	101/69	1.37	0.49-3.82	0.24	8/16	0.37	0.11-1.21	0.22

[a] Logistic regression models were adjusted for age, gender, race, education, smoking, family history of PD, and residential and occupational pesticide exposures.

[b] Overall Physical Activity (MET-h/wk) = 8*vigorous activity hour/week + 4*moderate activity hour/week; quartile cutoff.
Competitive Sports (MET-year) = sum of sport MET value*year of participation; tertile cutoff.
Occupational Activity (MET-year) = sum of job MET value*year of participation; quartile cutoff.

[c] Linear trend was tested using the midpoint of each exposure category as a continuous variable in the regression model.

Supplementary Table 2-S4. Lag analysis of multivariable-adjusted odds ratios (aOR) with 95% confidence intervals (CI) and p-for-trend for Parkinson's disease (PD) risk by competitive sports and occupational activity[a].

Cumulative physical activity [MET-year][b]	Exclusion of Last 10 years Prior to Diagnosis				Exclusion of Last 20 years Prior to Diagnosis			
	PD/Control	aOR	95%CI	P-for-trend[c]	PD/Control	aOR	95%CI	P-for-trend[c]
Competitive Sports								
Never	185/160	1 (Reference)			186/160	1 (Reference)		
<42	74/68	0.98	0.64-1.52		75/71	0.96	0.62-1.48	
42-105	52/52	0.93	0.57-1.54		54/54	0.94	0.57-1.54	
≥105	40/58	0.62	0.37-1.03	0.06	36/53	0.59	0.34-0.99	0.09
Occupational Activity								
< 56.7	111/113	1 (Reference)			140/149	1 (Reference)		
56.7-89.3	66/76	0.83	0.52-1.33		74/75	1.08	0.68-1.72	
89.3-130.8	79/75	0.88	0.52-1.49		73/59	1.05	0.61-1.80	
≥130.8	96/72	0.87	0.47-1.60	0.71	64/46	1.04	0.55-1.96	0.93

[a] Logistic regression models were adjusted for age, gender, race, education, smoking, family history of PD, and residential and occupational pesticide exposures.

[b] Competitive Sports (MET-year) = sum of sport MET value*year of participation; tertile cutoff.
Occupational Activity (MET-year) = sum of job MET value*year of participation; quartile cutoff.

[c] Linear trend was tested using the midpoint of each exposure category as a continuous variable in the regression model.

Supplementary Table 2-S5. Multivariable-adjusted odds ratios (aOR) with 95% confidence intervals (CI) and p-for-trend for Parkinson's disease (PD) risk and occupational activity, excluding highly occupational pesticide exposed individuals[a]

Occupational activity (MET-year)[b]	Men and Women				Men				Women			
	case/control	aOR	95% CI	p for trend[c]	case/control	aOR	95% CI	p for trend[c]	case/control	aOR	95% CI	p for trend[c]
< 56.7	88/85	1 (Reference)			11/11	1 (Reference)			77/74	1 (Reference)		
56.7-89.3	66/84	0.78	0.48-1.26		27/33	0.84	0.30-2.38		39/51	0.80	0.45-1.42	
89.3-130.8	80/79	0.91	0.54-1.56		57/57	1.14	0.42-3.10		23/22	1.09	0.51-2.35	
≥130.8	68/65	0.75	0.41-1.39	0.42	64/53	1.21	0.42-3.48	0.44	4/12	NA	NA	0.23

[a] Logistic regression model adjusted for age, gender, race, education, smoking, family history of PD, and residential and occupational pesticide exposures.

[b] Occupational Activity = sum of job MET value*year of participation (MET-year); quartile cutoff.

[c] Linear trend was tested using the midpoint of each exposure category as a continuous variable in the regression model.

Chapter 3. Physical Activity Modifies the Influence of APOE ε4 Allele and Type 2 Diabetes on Dementia and Cognitive Impairment among Older Mexican Americans

3.1 Abstract

Introduction: The etiologies of dementia are complex and influenced by genetic and environmental factors including medical conditions.

Methods: We used Cox regression model to estimate the individual and joint effects of physical activity (PA), apolipoprotein E (*APOE*) ε4 and diabetes status on risk of dementia and cognitive impairment without dementia (CIND) among 1,438 cognitively intact Mexican American elderly who were followed up to 10 years.

Results: The risk of developing dementia/CIND was increased more than threefold in *APOE* ε4 carriers or diabetics with low levels of PA compared with ε4 non-carriers or non-diabetics who engaged in high PA (ε4: hazard ratio [HR] 3.44, 95% confidence interval [CI] 1.85-6.39; diabetes: HR 3.11, 95% CI 1.87-5.18); the presence of all three risk factors increased risk by nearly 10-fold (HR 9.49 95% CI 3.57-25.3).

Discussion: Physical activity in elderly Hispanics protects strongly against the onset of dementia/CIND, especially in *APOE* ε4 carriers and those who have diabetes.

Keywords: Mexican American; Physical activity; Apolipoprotein E epsilon 4; Diabetes; dementia; Cohort study

3.2 Introduction

Cognitive decline and dementia risk are common in old age, and the number of people with dementia is projected to reach 115.4 million in 2050 worldwide [69]. In fast-growing aging populations, this will have a considerable impact on the healthcare and social systems; thus effective preventative public health strategies are needed. Evidence is accumulating that being physically active has profound effects on the brain's neurochemistry and plasticity and may protect against cognitive decline [21]. Indeed, a meta-analysis including 15 prospective cohort studies, with 30,331 non-demented participants, showed that high levels of physical activity (PA) at baseline versus sedentary lifestyle were associated with a decreased cognitive decline during follow-up by as much as 38% [16]. Moreover, many studies show that PA reduces the risk of cardiovascular disease, diabetes, hypertension and obesity, each contributing to cognitive impairment [70].

Genetic susceptibility may impact the effects of environmental factors [71]. While the apolipoprotein E (*APOE*) ε4 allele is a well-known genetic risk factor for Alzheimer's disease (AD) and dementia [72], several studies that examined interactions between PA and the *APOE* ε4 genotype reported inconsistent findings [73-78]. For example, a Finnish study found that high levels of PA in midlife were associated with lower risk of dementia/AD among *APOE* ε4 allele carriers [74], while in two other studies, late-life PA was inversely associated with dementia risk only among non-carriers in a US population and with risk of cognitive decline in Dutch male allele carriers [73, 75]. In addition, a recent German study (participants aged ≥ 75 years) suggested a possible additive interaction for the AD but not general dementia risk [78]. These inconsistencies may partially be due

to differences in study design or certain population characteristics such as diabetes status which has been proposed to possibly modify associations between *APOE* ε4 and AD or dementia [79].

Hispanics are the most rapidly growing segment of elderly living in the United States, but thus far very few studies have explored risk factors for dementia in this population [80-82]. Our prior work as well as other studies reported a higher prevalence of type 2 diabetes among Mexican Americans, and also suggested an increased risk of dementia/CIND among participants with diabetes [80]. While *APOE* ε4 carrier status was strongly associated with risk of AD and dementia, ε4 is less frequent among Mexican Americans [81, 82]. To date, no research however explored relationships between PA, *APOE* status, diabetes and cognitive impairment. We specifically focus on PA interactions because different from *APOE* status it is a modifiable behavioral factor that has been shown to prevent several chronic diseases and premature death even in old age [70]. Moreover, populations with a high proportion of individuals with multiple risk factors for cognitive decline might need special encouragement and culturally sensitive programs to remain physically active in older age.

3.3 Methods

Study population

All study participants were enrolled in the Sacramento Area Latino Study on Aging (SALSA) study, a large, prospective cohort study of community-dwelling Mexican Americans. Residents over 60 years of age at enrollment, resided in California's Sacramento Valley and self-designated as Latino/-a were eligible to enroll. A detailed description of sampling procedures has been published elsewhere [83]. The overall response rate was 85% for those contacted and about 22% of

the total eligible residents in Sacramento County were recruited; i.e. 1,789 aged 60-101 years were recruited and examined in 1998–1999 [83]. Cohort members were followed every 12–15 months via home visits during which clinical and cognitive assessments were conducted for up to seven times ending in 2008. In a semiannual 10-min phone call between home visits, we obtained updates on medications, health events, and some additional sociodemographic factors. Participants who 1) did not answer PA questions, or 2) did not provide either buccal or blood samples, or 3) had a diagnosis with dementia/CIND at baseline, or 4) did not participate in any follow-up visit were excluded from the analyses. A total of 1,438 participants are included in this analysis (Figure 1). SALSA has been approved by the Institutional Review Boards at the University of Michigan and the University of California at San Francisco and Davis and the University of North Carolina, Chapel.

Physical activity

At baseline, participants were asked to report the average number of hours they are spending on 18 different types of activities that are common among older adults during a regular week. We first assigned metabolic equivalents of task (MET) to each activity based on the Compendium of Physical Activities [61], and then multiplied this value with the reported time (hours per week) spent performing the activity (MET-hour/week). We generated moderate to vigorous cumulative PA measures by summing the MET-hour/week values over 8 activities that required a three-fold or more increase over the metabolic rate required by quiet sitting (≥3 METs); specifically walking, dancing, hunting or camping or boating, swimming or engaging in workouts, golfing or other moderate exercise, gardening or yard work, house repairs, and heavy housework.

APOE ε4 genotyping

Serum samples were collected from each participant and were taken to obtain deoxyribonucleic acid (DNA) for *APOE* analysis. *APOE* genotype was identified by polymerase chain reaction (PCR) amplification followed by restriction endonuclease digestion of the PCR product. Participants were considered *APOE* ε4 status positive if they carried at least one ε4 allele. The sequence surrounding the single nucleotide polymorphisms (SNPs) matched precisely the published sequences.

Diabetes

Diabetes status was based on reports of a physician diagnosis, antidiabetic medication use, or measured fasting glucose level ≥126 mg/dL (7.0 mmol/l), in a blood sample taken at the home visit (not only at baseline). In a medicine cabinet inventory, we recorded diabetes medications and classified them according to the Centers for Disease Control and Prevention Ambulatory Care Drug Database (http://www2.cdc.gov/drugs/); fasting glucose tests required no caloric intake for 8-hour or more. Because all participants were age 60 or above, we assumed that most were type 2 diabetes and we will refer to type 2 diabetes in this paper as such [84].

Dementia and Cognitive impairment without dementia (CIND)

Detailed procedures to screen and classify dementia and CIND were described elsewhere [83]. Briefly, each participant was assessed via two cognitive screening tests at baseline and each annual follow-up visit to determine whether they needed a neuropsychological evaluation: the Modified Mini-Mental State Examination (3MSE) and a delayed word recall trial from the Spanish English Verbal Learning Test (SEVLT). Participants were referred for a neuropsychological test battery [85] and a standard neuropsychological examination (Informant Questionnaire on Cognitive Decline in

the Elderly (IQCODE)) by a geriatrician if 1) their baseline scores on 3MSE or SEVLT fell below the 20th percentile, or 2) had ≥ 8-point decreased on the 3MSE or ≥ 3-point decreased SEVLT and the scores below the 20th percentile at follow-up [83]. A team of neurologists and a neuropsychologist reviewed all referred cases and classified them as demented, CIND, or cognitively normal on the basis of neuropsychological test battery and IQCODE as well as their history, mental status examination, and findings from the neurologic examination when available. Standard diagnostic criteria were applied for a diagnosis of dementia (DSM-IV) [86], Alzheimer disease (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association) [87], and vascular dementia (California Alzheimer's Disease Diagnostic and Treatment Centers) [88]. We combined dementia and CIND partly to improve our statistical power. Given that previous longitudinal studies have reported that people diagnosed with mild cognitive impairment (MCI) are more likely to develop dementia or AD than cognitively normal people [89], the combined outcome allowed us to include clinically important cognitive decline prior to dementia.

Other potential covariates

During the baseline interview, we asked participants to report their age, gender, education (years), country of birth, primary language, smoking status (never, current, or ever smoker), and alcohol use (never, daily, weekly, or monthly drinker). Trained interviewers measured participants' standing height and weight and obtained the body mass index (BMI; kg/m^2). Depressive symptoms were evaluated using Center for Epidemiologic Studies Depression Scale (range 0-60). Hypertension was based on measured systolic blood pressure (≥140 mmHg), self-report of a

physician diagnosis, and/or antihypertensive medication use. Information on vascular risk factors and diseases was obtained from self-reports of physician diagnoses such as stroke or myocardial infarction (MI). Morning fasting serum samples were used to test for low-density lipoprotein (LDL) cholesterol using the LDL Direct Liquid Select (number 7120; Equal Diagnostics). Statin use was derived from the medicine cabinet inventory [90].

Statistical analysis

Cox regression models with calendar time as the underlying time scale were used to assess the impact of PA, *APOE ε4* and diabetes on the risk of dementia/CIND. Participants who did not return for exams were censored at their last date of contact, or at their time of death if they died. We first categorized PA into tertiles based on the distribution of MET scores: <35 (low), 35-82.5 (medium), ≥82.5 (high) MET-h/wk. Since the effect estimates for the medium and high level of PA were found to be similar, we merged these into one category to increase power for our gene-environmental interaction (GxE) analyses. Age, sex, education, smoking status, history of stroke, and hours of standing or walking at work were entered into all models as covariates. The main effects of *APOE ε4* allele, diabetes status, and of PA were explored, together with all possible two- and three-way interactions with PA. For each variable included in the regression models, hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. We also calculated the relative excess risk due to interaction (RERI) to evaluate interactions on an additive scale [91, 92]. We further conducted analyses for dementia or CIND separately. We also adjusted for hypertension, cardiovascular disease, depressive symptoms, BMI, smoking status, alcohol use, nativity, the area of residence, type of occupation and hour of standing or walking at work as potential confounders.

Statistical analyses were performed using SAS 9.3. All analyses used an α level of 0.05 for statistical significance (two-tailed).

3.4 Results

Of 1,438 cognitively intact participants at baseline with at least one follow up, a total of 136 developed dementia/CIND during a mean follow-up time of 6.5 years. Table 1 represents the baseline characteristics of subjects by levels of PA. The proportion of subjects with at least one ε4 allele was slightly higher among participants with a high level of PA (14.7% vs. 12.8%), and no one in our population had ε2/ε2. Compared with participants who engaged in low levels of PA, those with higher activity levels were slightly younger, more often male, had more years of education and a lower prevalence of diabetes or history of stroke. They were also less likely to report ever having smoked cigarettes and more likely to be a former smoker.

The results of Cox regression analyses showed as expected that high levels of PA are inversely associated with risk of dementia/CIND, while *APOE* ε4 allele carrier status and diabetes at baseline increase risk (high PA: HR=0.70, P=0.04; ε4: HR=2.30, p<0.001; diabetes: HR=2.20, p<0.001; Table 2). Compared with non-carriers, having one and two ε4 alleles resulted in 2.08 and 11.5 fold increased risks of dementia/CIND, adjusting for multiple potential confounding factors.

When we examined combined effects of PA and *APOE* ε4 status, ε4-carriers who reported low levels of PA had a more than three-fold risk of dementia/CIND compared with highly active non-carriers (Table 3). Compared with physically active *APOE* ε4 non-carriers, we observed a 39% increase in risk for low PA non-carriers, a 120% higher risk for high PA ε4-carriers, and a

244% for low PA ε4-carriers. The same pattern was seen for combined effects of PA and diabetes. Compared with highly active non-diabetics, the HRs for dementia/CIND were 1.55, 2.39 and 3.11 for those reporting low PA, diabetes, and both, respectively. We further evaluated the combined associations of PA, *APOE* ε4 allele and diabetes with risk of dementia/CIND, and observed a nearly 10-fold risk increase for *APOE* ε4 carriers with diabetes and low levels of PA compared with those with high PA who were not diabetics and without a *APOE* ε4 allele (HR = 9.49, 95% CI = 3.57-25.3; Fig.1; Supplementary Table 1). No statistically significant two- or three-way super-additive interactions were found.

In sensitivity analysis, we adjusted for several other potential confounders (eg. nativity, hypertension, cardiovascular disease, depressive symptoms, BMI, smoking status, alcohol use, race, area of residence and type of occupation, LDL and statin) but estimates did not change substantially; thus, these factors were not included in final models. Analyses in which we considered dementia and CIND separately effect estimates were very similar to those we reported for the combined outcome (i.e. dementia/CIND) (Supplementary Tables 2-4).

3.5 Discussion

In this longitudinal study of Latino elderly adults, the co-presence of low levels of moderate to vigorous PA with either *APOE* ε4 status or diabetes was associated with a shorter dementia- and CIND-free follow-up time. Moreover, higher PA protected against the onset of dementia/CIND most strongly in those who were *APOE* ε4 carriers with diabetes.

A key finding of the current study is the large (nearly 10-fold) increased risk of dementia/CIND in *APOE* ε4 carriers who were physically inactive and also had diabetes as compared with non-ε4 carriers, non-diabetic and active individuals. The biological mechanisms underlying these associations are not known but may include inflammation and oxidative stress. Increasing evidence indicates that effects of *APOE* on oxidative stress prevention and anti-inflammation are isoform-specific [72, 93, 94]. Compared with other *APOE* isoforms, the ε4 allele is associated with higher oxidative stress and more likely to overactive pro-inflammatory and/or reduce anti-inflammatory responses [72, 93, 94]. Likewise, oxidative stress is one of the main mechanisms believed to explain insulin resistance in diabetes, and these cellular stresses are also related to inflammation [95]. Indeed, an increased cortical interleukin-6 (IL-6) level and more microvascular infarcts have been associated with risk of dementia in people with diabetes and biomarkers that are related to neuroinflammation [96]. Together, brain inflammation and oxidative stress may accumulate oxidative damage and have detrimental influences on neural tissue, which contribute to cognitive decline and AD [97]. Moreover, inflammation and pro-inflammatory cytokines are known to impair insulin-like growth factor 1 (IGF-1) transduction and brain-derived neurotrophic factor (BDNF) signaling in neurons, and low levels of IGF-1 and/or BDNF are associated with cognitive impairment [24]. Regular exercise is known to reduce visceral fat mass and adipose tissue, which contribute to systemic inflammation [45]. With or without fat mass loss, exercise induces IL-6 production from contracting muscles, and stimulates anti-inflammatory cytokines, such as IL-1 receptor antagonist (IL-1ra) and IL-10 while inhibiting tumor necrosis factor - alpha (TNF-α) production [24, 43, 98, 99]. In addition, regular PA can protect against chronic

metabolic and cardiorespiratory diseases known to be associated with an increased risk of cognitive decline [45, 100].

As has been reported previously [81, 82], *APOE* ε4 is less frequent among Mexican Americans. However, we found the AD/dementia prevalence to be similar to non-Hispanics, suggesting that other risk factors are more prevalent or that they may increase risk together with *APOE* ε4 [80, 81]. Prior studies of PA and *APOE* ε4 genotype and risk of dementia/AD reported conflicting results, which might have been due to risk modifying characteristics of the study population, or duration of follow-up, assessment and definition of PA and cognitive outcome, or the scale used to assess the interactions (i.e. additive or multiplicative interaction), and thus the comparisons across studies should be made with caution. In addition, previous studies generally presented data in questionnaire-specific categories (e.g. defining a high level of physical activity as participating in leisure-time activities several times a week) but did not provide personal energy expenditure estimates that can be translated into PA levels that correspond to guidelines. The Cardiovascular Health Study (CHS) is the only PA and *APOE* ε4 study that estimated personal energy expenditure (kilocalories/week) and used quartile categories [75]. The PA cutoff (≥35 MET-hours/week) in our study is slightly higher than this study's highest quartile but followed the Institute of Medicine (IOM) guidelines that recommend at least 60 minutes of moderate activity (approximately 3-6 METs) each day for "active" adults [101]. Our results corroborate previous findings that - regardless of *APOE* ε4 status - being active in late-life benefits cognition [77, 78, 102].

Our findings agree with prospective population-based studies and a meta-analysis indicating that people with diabetes are at increased risk of dementia [103]. However, two studies that recruited

Mexican Americans did not support these findings despite the increased prevalence of diabetes [81, 82]. When we examined the combined effects of diabetes and PA, we estimated 3-fold increased risks in diabetics with low levels of PA compared with non-diabetics with high PA levels. To our knowledge, no studies have yet reported on interactions between PA and diabetes for dementia or cognition decline. Nevertheless, a randomized controlled trial suggested that 6 months of aerobic exercise improved executive function and insulin sensitivity in people with prediabetes or newly diagnosed type 2 diabetes [104]. We also observed a 4.26 times higher risk for dementia/CIND among *APOE* ε4 carriers with diabetes, compared with participants who had neither diabetes nor *APOE* ε4; however, we did not find an interaction on an additive scale (Supplementary Table 5). While the 2 US studies reported a small but super-additive interaction, for AD they estimated 4-fold joint risks similar in size for diabetes and *APOE* ε4 [79].

SALSA is a longitudinal population-based study cohort of older people of Mexican heritage (N=1,789) from 6 county areas that included the metropolitan area as well as surrounding rural counties. This is the only population-based study that assessed dementia in Mexican Americans, a large but understudied ethnic group. The repeated-measures design assessing cognition every 12–15 months for up to seven study visits, enabled us to study incident dementia/CIND over a relatively long period (on average 6.5 years) and assess the influence of baseline PA, diabetes and *APOE* ε4 allele carrier status. Furthermore, we created cumulative PA scores (MET-hour/week) to take both intensity and duration into account, thus measuring PA more comprehensively than prior studies that only presented PA data in specific categories such as whether the participant engaged in leisure-time activities several times a week. Finally, we also assessed and were able to

adjust for a large number of demographic and health-related covariates i.e. potential confounders. We previously reported that statin users were less likely to develop dementia/CIND [90], however, further adjusting for baseline statin use in the model did not change the results. In addition, we omitted to adjust for LDL since it can be considered an intermediate in the pathway between PA and cognitive function.

Limitations include that we were unable to assess and/or adjust for changes of PA in relation to cognitive decline because we only collected PA once at baseline. Additionally, our PA measure based on self-reported information is inevitably prone to reporting errors. We did not evaluate the reliability of PA assessment in our SALSA population, however, the PA questionnaire originated from the Alameda County Study [105] and a large number of previous studies found associations between this PA measure and various health outcomes; external validity and internal reliability are high. This measure is also very similar to the Minnesota Leisure Time Physical Activity Questionnaire, which is commonly used among the elderly and has acceptable reliability and validity [106, 107]. Moreover, given that dementia and cognitive impairment occurred during follow-up, it is reasonable to suggest that misclassification of PA level measured at baseline is non-differential and most likely biases our results toward the null. We combined dementia and CIND into one outcome (i.e. dementia/CIND) for purposes of parsimony. Although prior studies reported conflicting associations of PA and *APOE* ε4 with the risk of dementia, AD and cognitive decline, we observed a consistent pattern of associations for all three definitions i.e. for dementia and CIND combined as well as separately (Supplementary Table 2). The 2-way and 3-way interaction results are also consistent in direction and significance for both the combined and separate outcomes

(Supplementary Table 3&4). As expected, the smaller cell sample sizes for a single outcome produce wider confidence limits. While we adjusted for a number of demographic and comorbidity-related factors, as in all observational studies, residual confounding is a possibility. Although associations between PA and depression have been reported [108], and depression may be a risk factor for dementia [109], we did not observe an interaction of PA and depression on dementia/CIND risk, and baseline depressive symptoms did not confound our results, similar to previous studies [74, 75, 81]. Also, we cannot rule out the possibility that low PA is a proxy of other adverse health outcomes that contributes to cognitive decline. Lastly, compared with healthier participants, those with diabetes or cognitive decline may be more likely to be lost to follow-up. However, our attrition rate was low (5%) and the expected effects of this drop out would have been a bias towards the null.

In conclusion, Mexican Americans who are physically inactive and are *APOE* ε4 allele carriers with diabetes were nearly 10 times more likely to have incident dementia/CIND than active, non-diabetics who do not carry the *APOE* ε4 allele. In light of this rapidly growing elderly population with a very high rate of diabetes, it might be worthwhile targeting *APOE* ε4 carriers with diabetes for programs that increase PA as an effective preventive strategy against cognitive impairment.

3.6 Figure and Tables

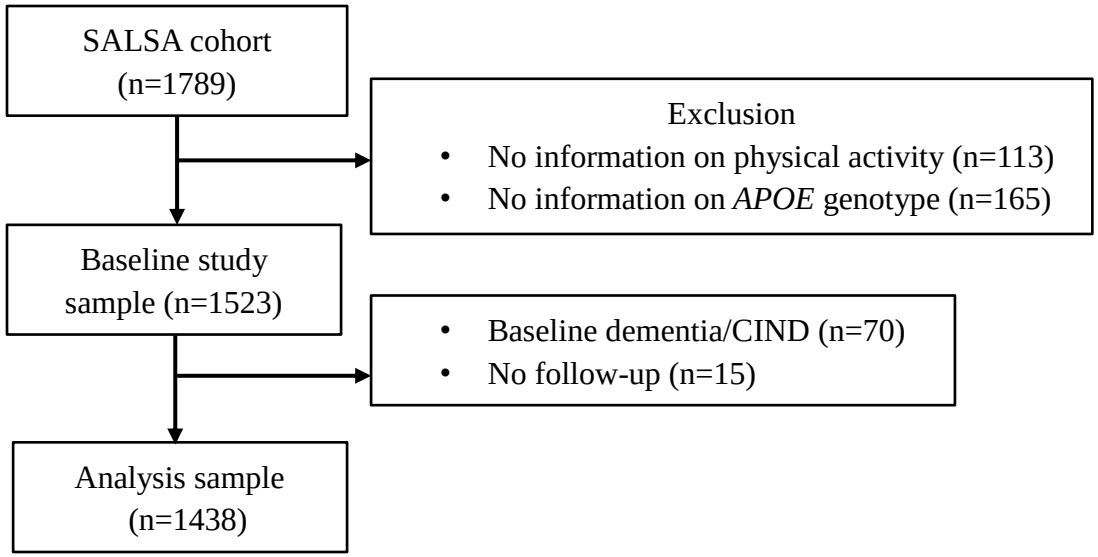


Figure 3-1. Flowchart of study participants, Sacramento Area Latino Study on Aging (SALSA), 1998-2008

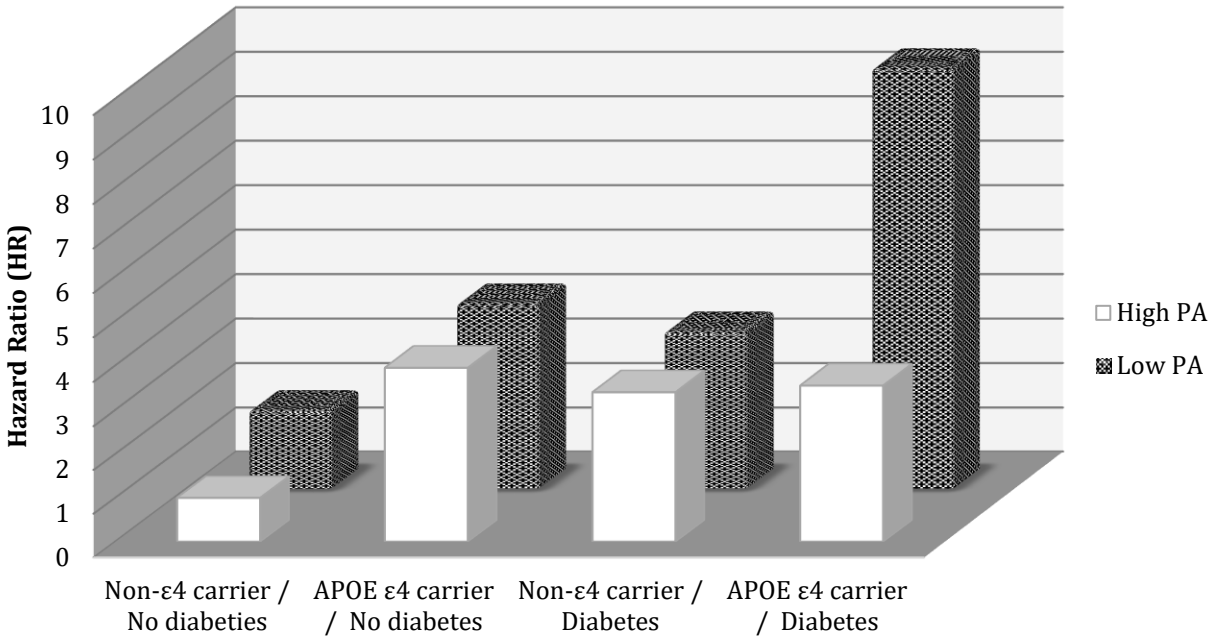


Figure 3-2. Three-way interactions of physical activity (PA), apolipoprotein E (*APOE*) ε4 allele and diabetes status at baseline on the risk of dementia and cognitive impaired without dementia (CIND).

Hazard ratios adjusted for gender and baseline age (years), education, smoking status, history of stroke, and hours of standing or walking at work; relative excess risk due to interaction (RERI; 95% CI) = 2.34 (-7.03-22.1).

Table 3-1. Sample characteristics of the study population at baseline by physical activity (PA), Sacramento Area Latino Study on Aging, 1998-2008

	High PA (n=897)		Low PA (n=541)	
Variable	mean (SD)	n (%)	mean (SD)	n (%)
Demographic				
Age, year	69.7 (6.2)		70.5 (7.2)	
Male		421 (46.9)		177 (32.7)
Education, year	7.7 (5.4)		7.1 (5.3)	
Born in Mexico		392 (43.7)		243 (44.9)
Reside in urban area		777 (86.6)		471 (87.1)
Health-related factors at baseline				
Cardiovascular disease		297 (33.1)		214 (39.6)
Hypertension		600 (66.9)		389 (71.9)
Diabetes		261 (29.1)		195 (36.0)
Stroke		56 (6.2)		57 (10.5)
LDL-C, mg/dl	125.2 (35.0)		119.4 (34.0)	
Statin at baseline		70 (7.8)		58 (10.7)
Behavioral				
Alcohol				
Frequent (daily) drinker		88 (9.8)		38 (7.0)
Moderate (weekly) drinker		129 (14.4)		30 (5.6)
Occasional (monthly) drinker		103 (11.5)		38 (7.0)
Yearly/rarely/never drinker		577 (64.3)		434 (80.4)
Smoking status				
Current		104 (11.6)		57 (10.5)
Former		390 (43.5)		220 (40.7)
Never		403 (44.9)		64 (48.8)
Depression (CESD)	8.6 (9.2)		11.4 (11.7)	
Occupation				
Non-manual		206 (23.2)		106 (19.7)
Manual		550 (62.0)		299 (55.6)
Other (housewives/unemployed)		131 (14.8)		133 (24.7)
Cognitive performance				
3MSE	87.5 (9.4)		85.4 (11.6)	
SEVLT (No. of words)	9.0 (2.8)		8.5 (2.9)	
***APOE* status**				
Any ε4		132 (14.7)		69 (12.8)
ε2/ε3		65 (7.3)		45 (8.3)
ε2/ε4		7 (0.8)		2 (0.4)
ε3/ε3		700 (78.0)		427 (78.9)
ε3/ε4		116 (12.9)		65 (12.0)
ε4/ε4		9 (1.0)		2 (0.4)

Abbreviation: SD, standard deviation; CESD, Center for Epidemiologic Studies Depression Scale; 3MSE, Modified Mini-Mental State Examination; SEVLT, delayed word recall trial from the Spanish English Verbal Learning Test; *APOE*, apolipoprotein E.

[a] PA cutoff = 35 MET-hour/week

Table 3-2. Cox proportional hazards regression to evaluate the risk of dementia/CIND according to physical activity (PA), *APOE* ε4 allele and diabetes status, controlled for baseline covariates

	Model 1		Model 1		Model 2		Model 2	
	Adjust HR (95% CI)	p-value	Adjust HR (95% CI)	p-value	Adjust HR (95% CI)	p-value	Adjust HR (95% CI)	p-value
Physical activity, high vs low	0.66 (0.47-0.92)	0.02	0.64 (0.46-0.91)	0.01	0.70 (0.49-0.99)	0.04	0.68 (0.48-0.97)	0.03
APOE ε4 allele (yes vs. no)	2.15 (1.43-3.22)	<.001			2.30 (1.53-3.47)	<.001		
APOE ε4 allele (1 vs 0)			1.95 (1.27-2.98)	<.001			2.08 (1.36-3.20)	<.001
APOE ε4 allele (2 vs 0)			9.52 (3.42-26.5)	<.001			11.5 (4.08-32.2)	<.001
Diabetes	2.41 (1.71-3.38)	<.001	2.45 (1.74-3.44)	<.001	2.20 (1.56-3.12)	<.001	2.24 (1.58-3.17)	<.001
Age	1.13 (1.10-1.15)	<.001	1.13 (1.10-1.16)	<.001	1.12 (1.09-1.14)	<.001	1.12 (1.09-1.15)	<.001
Male vs. female	0.77 (0.54-1.10)	0.15	0.76 (0.53-1.09)	0.14	0.80 (0.54-1.18)	0.25	0.80 (0.54-1.18)	0.26
Education, year					0.96 (0.92-0.99)	0.01	0.96 (0.92-0.99)	0.01
Former smoker vs never					0.94 (0.63-1.38)	0.74	0.92 (0.62-1.36)	0.68
Current smoker vs never					1.88 (1.10-3.23)	0.02	1.92 (1.12-3.29)	0.02
Stroke					1.89 (1.19-3.02)	0.01	1.92 (1.20-3.07)	0.01
Hours of standing or walking at work					0.95 (0.91-1.00)	0.05	0.95 (0.91-1.00)	0.05

Abbreviation: CIND, cognitive impairment without dementia; *APOE*, apolipoprotein E; HR, hazard ratio; CI, confidence interval.
[a] PA cutoff = 35 MET-hour/week

Table 3-3. Joint effects between physical activity (PA) and *APOE* ε4 allele or diabetic status on incident dementia/CIND [a]

	High level of PA [b]			Low level of PA [b]		
	case/total	Crude HR (95% CI)	Adjust HR (95% CI)	case/total	Crude HR (95% CI)	Adjust HR (95% CI)
APOE ε4						
No	52/765	1.00	1.00	52/432	1.79 (1.22-2.63)	1.39 (0.94-2.07)
Yes	19/172	1.96 (1.16-3.32)	2.20 (1.29-3.74)	13/69	2.80 (1.53-5.15)	3.44 (1.85-6.39)
Diabetes						
No	37/636	1.00	1.00	38/346	2.07 (1.32-3.26)	1.55 (0.97-2.48)
Yes	34/261	2.75 (1.73-7.39)	2.39 (1.49-3.84)	27/195	3.13 (1.91-9.15)	3.11 (1.87-5.18)

Measure of interaction on additive scale: RERI (95% CI): PA and ε4 = 0.85 (-1.34-3.04); PA and diabetes = 0.16 (-1.40-1.73)

Abbreviation: *APOE*, apolipoprotein E; CIND, cognitive impairment without dementia; HR, hazard ratio; CI, confidence interval; RERI, relative excess risk due to interaction.

[a] Cox proportional model adjusted for gender and baseline age (years), diabetes or *APOE* ε4, education, smoking status, history of stroke and hours of standing or walking at work.

[b] PA cutoff = 35 MET-hour/week

3.7 Supplement

Supplementary Table 3-S1. Effect estimates (HRs and 95% CI) of interactions of physical activity (PA), *APOE* ε4 allele and diabetic status for incident dementia/CIND [a]

	High level of physical activity [b]			Low level of physical activity [b]		
APOE ε4 /Diabetes	case/total	Crude HR (95% CI)	Adjust HR (95% CI)	case/total	Crude HR (95% CI)	Adjust HR (95% CI)
No/No	24/534	1.00	1.00	30/298	2.48 (1.45-4.24)	1.80 (1.04-3.13)
Yes/No	13/102	2.74 (1.39-5.38)	3.95 (2.00-7.80)	8/48	3.76 (1.69-8.36)	4.20 (1.86-9.48)
No/Yes	28/231	3.36 (1.95-5.81)	3.40 (1.96-5.90)	22/174	3.70 (2.07-6.60)	3.57 (1.97-6.46)
Yes/Yes	6/30	4.72 (1.93-11.54)	3.55 (1.45-8.70)	5/21	6.67 (2.54-17.49)	9.49 (3.57-25.25)

Abbreviation: HR, hazard ratio; CI, confidence interval; *APOE*, apolipoprotein E; CIND, cognitive impairment without dementia.

[a] Cox proportional model adjusted for gender and baseline age (years), education, smoking status, history of stroke and hours of standing or walking at work.

[b] PA cutoff = 35 MET-hour/week

Supplementary Table 3-S2. Cox proportional hazards regression to evaluate the risk of dementia or CIND according to physical activity (PA), *APOE* ε4 allele and diabetes status, controlled for baseline covariates

	Dementia (N=94/1482) [b]		CIND (N=66/1464) [b]	
Parameter	Adjust HR (95% CI)	p-value	Adjust HR (95% CI)	p-value
Physical activity, high vs low [a]	**0.77 (0.51-1.17)**	**0.22**	**0.59 (0.36-0.97)**	**0.04**
***APOE* ε4 allele (yes vs. no)**	**2.30 (1.40-3.77)**	**<.001**	**2.29 (1.30-4.02)**	**<.001**
Diabetes	**2.37 (1.56-3.58)**	**<.001**	**2.23 (1.36-3.66)**	**<.001**
Age	1.13 (1.10-1.16)	<.001	1.09 (1.05-1.13)	<.001
Male vs. female	0.65 (0.41-1.04)	0.08	0.89 (0.50-1.56)	0.67
Education, year	0.97 (0.93-1.01)	0.18	0.96 (0.92-1.01)	0.13
Former smoker vs never	1.16 (0.73-1.85)	0.52	0.74 (0.42-1.32)	0.31
Current smoker vs never	2.14 (1.09-4.20)	0.03	1.25 (0.56-2.82)	0.59
Stroke	2.37 (1.40-4.02)	0.00	1.01 (0.46-2.24)	0.98
Hours of standing or walking at work	0.88 (0.75-1.04)	0.13	0.97 (0.92-1.02)	0.23

Abbreviation: CIND, cognitive impairment without dementia; *APOE*, apolipoprotein E; HR, hazard ratio; CI, confidence interval.

[a] PA cutoff = 35 MET-hour/week
[b] N = incident case/total number

Supplementary Table 3-S3. Joint effects between physical activity (PA) and APOE ε4 allele or diabetic status on incident dementia or CIND [a]

	Dementia		CIND	
	case/total	Adjusted HR (95% CI)	case/total	Adjusted HR (95% CI)
No *APOE* ε4 allele/High PA[b]	36/786	1.0	22/772	1.0
APOE ε4 allele/High PA[b]	13/136	1.94 (1.06-3.57)	10/135	2.25 (1.21-4.17)
No *APOE* ε4 allele/Low PA[b]	36/488	1.40 (0.91-2.16)	27/484	1.42 (0.91-2.22)
APOE ε4 allele/Low PA[b]	9/72	2.69 (1.35-5.38)	7/73	3.27 (1.66-6.47)
No diabetes/High PA[b]	22/649	1.0	18/643	1.0
Diabetes/High PA[b]	27/273	2.95 (1.66-5.23)	14/264	2.10 (1.04-4.25)
No diabetes/Low PA[b]	26/355	1.63 (0.91-2.94)	18/356	1.60 (0.82-3.12)
Diabetes/Low PA[b]	19/205	3.02 (1.60-5.70)	16/201	3.79 (1.91-7.54)

Abbreviation: *APOE*, apolipoprotein E; CIND, cognitive impairment without dementia; HR, hazard ratio; CI, confidence interval.

[a] Cox proportional model adjusted for gender and baseline age (years), diabetes or *APOE* ε4, education, smoking status, history of stroke and hours of standing or walking at work.

[b] PA cutoff = 35 MET-hour/week

Supplementary Table 3-S4. Effect estimates (HRs and 95% CI) of interactions of physical activity (PA), APOE ε4 allele and diabetic status for incident dementia or CIND [a]

	High level of physical activity [b]				Low level of physical activity [b]			
	Dementia		CIND		Dementia		CIND	
APOE ε4 /Diabetes	case/total	Adjusted HR (95% CI)	case/total	Adjusted HR (95% CI)	case/total	Adjusted HR (95% CI)	case/total	Adjusted HR (95% CI)
No/No	14/545	1.0	12/540	1.0	19/304	1.84 (0.91-3.75)	14/305	1.84 (0.84-4.04)
Yes/No	8/104	4.27 (1.77-10.3)	6/103	3.16 (1.18-8.49)	7/51	5.54 (2.20-14.0)	4/51	3.52 (1.12-11.0)
No/Yes	22/241	4.24 (2.15-8.38)	10/232	2.44 (1.05-5.67)	17/184	3.91 (1.89-8.11)	13/179	4.21 (1.90-9.35)
Yes/Yes	5/32	4.75 (1.71-13.3)	4/32	4.71 (1.51-14.7)	2/21	5.64 (1.27-25.1)	7/22	9.28 (2.57-33.6)

Abbreviation: *APOE*, apolipoprotein E; CIND, cognitive impairment without dementia; HR, hazard ratio; CI, confidence interval.

[a] Cox proportional model adjusted for gender and baseline age (years), education, smoking status, history of stroke and hours of standing or walking at work.

[b] PA cutoff = 35 MET-hour/week

Supplementary Table 3-S5. Joint effects between APOE ε4 allele and diabetic status on incident dementia/CIND [a]

	No diabetes at baseline			Diabetes at baseline		
	case/total	Crude HR (95% CI)	Adjust HR (95% CI)	case/total	Crude HR (95% CI)	Adjust HR (95% CI)
APOE ε4						
No	59/861	1.0	1.0	57/424	2.34 (1.59-3.44)	2.71 (1.82-4.02)
Yes	21/152	2.04 (1.23-3.38)	3.00 (1.78-5.04)	11/52	3.64 (1.90-6.96)	4.26 (2.20-8.24)

Measure of interaction on additive scale: RERI (95% CI): -0.73 (-3.59-2.14)

Abbreviation: *APOE*, apolipoprotein E; CIND, cognitive impairment without dementia; HR, hazard ratio; CI, confidence interval; RERI, relative excess risk due to interaction.

[a] Cox proportional model adjusted for gender and baseline age (years), physical activity, education, smoking status, history of stroke and hours of standing or walking at work.

Chapter 4. The role of Physical Activity and Inflammation for Mortality, Cognition, and Depression in older Mexican Americans

4.1 Abstract

Importance: Physical activity (PA) is associated with a decreased risk of mortality, dementia and depression, yet the mechanism is not well understood and the association is understudied in Mexican Americans.

Objective: To examine (1) the associations between PA and mortality, dementia/cognitive impairment without dementia (CIND), or depression among elderly Mexican American; and (2) whether plasma inflammatory markers explain the associations.

Design, Setting and Participants: The prospective cohort of Sacramento Area Latino Study on Aging (SALSA) study was conducted from 1998–1999 through 2008 and followed for mortality until 2016. The study compromised 1,459 community-dwelling participants self-designated Latino and aged 60-101 years at baseline residing in a six-county area in California's Sacramento Valley.

Exposures: PA was assessed from self-reported average hours participating in 18 activities in a regular week at baseline and transformed into weekly Metabolic Equivalent (MET) values.

Main Outcomes and Measures: Demographic and inflammatory biomarkers were measured at baseline; cohort members were followed every 12–15 months via home visits for up to seven times. Cox proportional hazards regression was used to evaluating associations of baseline PA level with mortality, dementia/CIND or depression and mediating effects of inflammatory markers were estimated in additive hazard models.

Results: The mean (SD) age of participants at baseline was 70.1 (6.6) years and 607 (41.4%) were men. A total of 489 participants died, 129 developed dementia/CIND and 398 depression during follow-up. Low level of PA (<35 MET-hour/week) was associated with increased mortality,

incident dementia/CIND, and incident depression with HRs of 1.40 (1.17-1.68), 1.37 (95% CI, 0.96-1.96) and 1.23 (1.00-1.52). Being inactive/ less active added 512 (95% CI, 23-212) events of dementia/CIND per 100 000 person-years (direct effect), while through a mediating path interleukins 6 (IL-6) added another 49 (95% CI, 5-94) cases, or 9% of the total effect. For mortality, 7-8% of the total effect of PA was mediated through IL-6, tumor necrosis factor - alpha (TNF-α) or TNF-α receptors, but none of the inflammatory markers mediated the PA effects on depression. **Conclusions and Relevance:** Our results not only confirm previous findings that being active even in older age protects against neuropsychiatric and degenerative disorders and all-cause mortality, but also suggests that plasma inflammatory markers (especially IL-6 and TNF- α) may partly explain the protection of PA against dementia/CIND and mortality.

4.2 Background

Dementia and depression are the most common neuropsychiatric/degenerative disorders in old age, representing a large social and economic burden globally.[110] With the rapid growth of the elderly population, prevention of aging-related dementia and late-life depression is of growing importance. Epidemiological evidence is accumulating that regular physical activity (PA) and active lifestyle has many health benefits, including lowering mortality, preventing some major chronic diseases, and improving physical, psychological, and social functioning.[111] While it has been shown that exercise induces an anti-inflammatory response that is thought to be a key mechanism for reducing atherosclerosis and protecting against metabolic dysregulation,[45] much less is known whether this mechanism is also responsible for the influence exercise has on brain function including cognition and depression.

Transient inflammation is a protective response that stimulates healing processes after injury and infection, yet failure to completely terminate the immune response within an adequate timeframe may cause a systemic state of low-grade chronic inflammation that may also contribute to dementia and depressive disorders in the elderly.[43, 44, 112-114] Several studies have found elevated levels of C-reactive protein (CRP), interleukins 6 (IL-6) and/or tumor necrosis factor - alpha (TNF-α) to be related with cognitive deterioration.[43, 113, 115] Recent meta-analyses also supported associations between depression and peripheral elevation of inflammatory cytokine levels and suggested inflammation as indicated by CRP and IL-6 leading to depression.[116-118] On the other hand, inverse associations between physical activity and markers of chronic inflammation, reflected by increased CRP concentrations and other pro-inflammatory cytokines have also been reported.[43, 119, 120] However, no prior studies have examined whether inflammatory markers mediate the association between physical activity and cognition or mood. The main aim of this

paper is to examine whether CRP, IL-6 or TNF-α may explain associations between physical activity and dementia/cognitive impairment without dementia (CIND), or depression, and –finally - mortality among elderly Mexican American (Fig. 1).

4.3 Methods

Study population

The Sacramento Area Latino Study on Aging (SALSA) is a large, prospective cohort of community-dwelling Mexican Americans who resided in California's Sacramento Valley and self-designated as Latino/-a. A detailed description of sampling procedures has been published elsewhere.[83] Briefly, this study population consisted of 1,789 individuals aged 60-101 years recruited in 1998–1999. The overall response rate was 85% for those contacted and about 22% of the total of all eligible residents were recruited. Cohort members were followed every 12–15 months via home visits during which clinical and cognitive assessments were conducted for up to seven times ending in 2008; in a semiannual 10-min phone call between home visits, we obtained updates on medications, health events, and some additional sociodemographic factors. The annual attrition rate was 5%, including mortality and loss to follow up between baseline and 2008.[121]

For this analysis, we excluded individuals who met 1 or more of the following criteria: missing data for physical activity (N=113) or inflammatory markers (N=227); no follow-up data (N=92); prevalent dementia/CIND or depression at baseline for the analyses in which we target dementia/CIND or depression. Ultimately, a total of 1,459 participants constituted the final sample size for mortality, 1,397 for incident dementia/CIND and 935 for incident depression. All participants gave informed consent to participate in the study; ethical approval was obtained from

the Institutional Review Boards at the University of Michigan and the University of California at San Francisco and Davis.

Measures

Physical activity — Baseline physical activity information was assessed from self-report as previously described.[122] Participants were asked to report their average hours per week participating in 18 different types of common activities among older adults in a regular week. Eight activities that required a three-fold or more increase over the metabolic equivalent (MET) required by quiet sitting (≥3 METs) were summed to generate weekly moderate to vigorous physical activity measures (MET-hour/week); participants were classified into "low" or "high" PA group based on the first tertile (<35 MET-hour/week, ≥35 MET-hours/week), following the guidelines from the Institute of Medicine.[101]

Ascertainment of Mortality — Deaths among participants were identified by 1) interviews with family members when tracking participants who could not be reached for annual home visits or interim 6-months phone follow-ups, 2) online surveillance of death notices, and 3) review of the Social Security Death Index, the National Death Index, and vital statistics data files from the state of California between 1998 and 2016. We have complete or partial social security numbers on 80% of the deceased participants and 93.1% have been linked to death certificates with information on the cause of death. The cause of death was classified by the International Classification of Diseases, Tenth Revision.

Dementia and Cognitive impairment without dementia (CIND) — Procedures to classify dementia and CIND were extensively described elsewhere.[83] Briefly, at baseline and each annual follow-up visit, participants underwent 2 cognitive evaluations (the Mini-Mental State

Examination (3MSE) and a delayed word recall trial from the Spanish English Verbal Learning Test (SEVLT)) to determine whether they needed further neuropsychological testing. If the scores on 3MSE or SEVLT fell below the 20th percentile (≤77 3MSE or ≤5 SEVLT) or decreased from baseline by ≥8-points on the 3MSE or by ≥3-points on the SEVLT, the participants were referred for a neuropsychological test battery and a standard neuropsychological examination done by a geriatrician. A team of neurologists and a neuropsychologist first reviewed all referred participants and classified them as demented, CIND, or cognitively normal based on the standard diagnostic criteria for dementia (DSM-IV),[86] Alzheimer disease (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association),[87] and vascular dementia (California Alzheimer's Disease Diagnostic and Treatment Centers);[88] those who were diagnosed with dementia and CIND cases were then referred for magnetic resonance imaging and appropriate laboratory tests. Participants who were not diagnosed during follow-up as having dementia or CIND but died prior to an evaluation were reclassified if any of the following causes of death were listed on the death certificate: dementia of Alzheimer type, vascular dementia, other dementia, or unspecified dementia.

Depression — We used the Center for Epidemiologic Studies Depression Scale (CES-D) at baseline and each follow-up to evaluate the depressive symptoms. CES-D consists of 20 self-reported items designed to measure depressive symptoms experienced during the previous week, and has been widely used and validated in older adults.[123] The generally agreed upon the cut-off score of ≥16 symptoms or using an anti-depressant prescription drug were used to define depression.[124, 125] Antidepressant drug use was derived from a medicine cabinet inventory; these medications consisted predominantly of selective serotonin reuptake inhibitors and tricyclic antidepressants, with fewer taking atypical antipsychotics, noradrenergic and specific serotonergic

antidepressants, norepinephrine–dopamine reuptake inhibitors, and serotonin-norepinephrine reuptake inhibitors.

Inflammatory Markers — Baseline serum samples were collected from each participant and processed/stored at the Medical Center Clinical Laboratory at the University of California, Davis. hs-CRP levels were measured with the CRP Ultra Wide Range Reagent Kit latex-enhanced immunoassay (formerly Equal Diagnostics, Exton, PA); measures 0 - 160 mg/L. IL-6 and TNF-α and their receptors were determined by using the Quantiglo Chemiluminescent Immunoassay, Q60000B (IL-6, range of 0.5 – 200 pg/mL), QTA00B (TNF-α, range of 1.1 - 560 pg/mL), DR600 (IL-6 receptor, range of 31.2 – 2000 pg/mL), DRT100 (TNF-α receptor 1, range of 1.1 - 560pg/mL) and DRT200 (TNF-α receptor 2, range of 7.8 – 500 pg/mL) respectively (R&D Systems, Minneapolis, Minnesota). For each inflammatory marker, we dichotomized using the upper quartile (Q3) cutoff of this marker among participants having baseline hs-CRP level less than 10 mg/L, assuming that CRP ≥10 mg/L indicates clinically relevant inflammation.

Other covariates — During the baseline interview participants reported their age, sex, education (years) and smoking status (never, current, ever). Body mass index (BMI) was calculated using measured height and weight (kg/m^2). To control for the effect of chronic health conditions on mortality and other outcomes, a modified Charlson comorbidity index was created by assigning a point each for a history of myocardial infarction, congestive heart failure, stroke, dementia, liver disease, diabetes, renal disease, any malignancy, and leukemia or lymphoma and summing across these items.[126]

Statistical analysis

We summarized the participants' characteristics according to their baseline level of PA. The associations between physical activity and mortality, dementia/CIND, or depression were assessed separately using Cox proportional hazards regression models, adjusted for participants' age, gender, education, BMI, smoking and modified Charlson index. Mediation analysis was performed to determine whether inflammatory markers at least partly explain associations between physical activity and these outcomes. Given that the assumption of Cox model cannot be satisfied in mediation analysis and the hazard ratios (HR) cannot be related to absolute numbers of events,[127] we also employed the Aalen additive hazard model to assess direct and indirect effect in a survival analysis setting.[127, 128]

We applied a marginal structural modeling approach based on a counter-factual framework to estimate the natural direct effect (DE) of baseline PA on mortality, the risk of dementia/CIND or depression and the natural indirect effect (IE) of baseline PA via the inflammatory pathway with biomarkers as potential mediators.[128, 129] (Fig. 1) Briefly, we first conducted logistic regression analysis to estimate the effects of PA on each of the inflammatory markers (mediator) adjusted for all confounding factors. Next, we created a new data set by duplicating each original observation and generating additional counterfactual exposure variables (i.e. assigning the opposite of the observed PA value) in the extended dataset. Weights were derived from the logistic regression models by regressing the dichotomized inflammatory markers on PA and confounding factors. The final step was to estimate the effect of both PA levels (low vs. high) and of each inflammatory marker on mortality, the onset of dementia/CIND or depression by fitting the additive hazards model and adjusting for baseline confounders. These estimates can be interpreted as the number of additional events per 100,000 person-years at risk, when comparing low with high PA subjects to generate the DE and IE of baseline PA on each of outcomes. The proportion mediated is given

by the ratio of the estimated indirect to the total effect. Confidence intervals for the DE and the IE were extracted directly from the model, while 95% CIs for the mediated proportion were computed by bootstrapping using 10,000 samples. Due to the mutual dependence between all inflammatory markers conditional on exposure (PA) and covariates, we did not consider all inflammatory markers in the same model but relied on one model for each marker.

Sensitivity analyses were conducted removing deaths, dementias/CINDs or depressions in the first 1-year or 2-year of follow up. To assess the interaction between PA and inflammatory markers on the multiplicative scale, we also added a PA × biomarker product term in the model for each inflammatory marker, and calculated the relative excess risk for interaction (RERI) as a measure of additive interaction.[91] Mediation analysis was carried out using R 3.4.2 and all other analyses were conducted using SAS 9.4 (SAS Institute Inc., USA). Effect estimates may be considered formally statistically significant if the null value is not included in the 95% CI we are showing.

4.4 Results

In terms of baseline characteristics of participants (Table 1), those who were inactive or less active were more likely to be female, smokers, and had a higher BMI, the prevalence of chronic diseases, and levels of inflammatory markers. During follow-up, a total of 489 participants died, 129 developed dementia/CIND and 398 depression. Low levels of physical activity were associated with increased mortality, incident dementia/CIND, and incident depression with HRs of 1.46 (95% CI, 1.22-1.75), 1.45 (95% CI, 1.02-2.06) and 1.23 (95% CI, 1.00-1.51) after adjusting for age, gender, and education (Table 2). The effect estimate for physical activity decreased slightly for dementia/CIND to 1.37 (95% CI, 0.96-1.96) after further adjustment for smoking, BMI, and Charlson index. While all associations remained unchanged in the first year lagged analyses for

dementia/CIND, depression and mortality (supplementary table 1), the effect estimate for physical activity and depression moved towards the null after we excluded 129 depression cases that occurred in early follow-up (2 years after baseline) (HR, 1.15 [95% CI, 0.89-1.49]).

We examined mediation through inflammation by adding inflammatory biomarkers into each outcome models. (Table 3 and 4). Specifically, comparing inactive/less active participants to active ones, we estimated that around 10% of the total effect was mediated through IL-6, TNF-α, TNF-α receptor 1 or TNF-α receptor 2. In absolute terms, 97 (95% CI, 15-180) additional deaths per 100,000 person-years at risk were mediated through changes in IL-6, 100 deaths (95% CI, 27-173) through TNF-α, 98 deaths (95% CI, 4-191) through TNF-α receptor 1 and 118 deaths (95% CI, 23-212) through TNF-α receptor 2. In terms of direct effect for dementia/CIND, being inactive/ less active added 512 (95% CI, 23-212) events per 100,000 person-years, and we also observed an IE through IL-6 that added another 49 (95% CI, 5-94) dementia/CIND cases per 100 000 person-years, or 9% of the total effect. For depression, the direct effects for PA as well as the mediated effects we observed in models adjusted for age, gender and education especially for IL-6 and TNF-α receptors disappeared after further co-adjusting for BMI, smoking and modified Charlson index.

Supplementary table 5 displays the PA – inflammation interaction in relation to mortality, dementia/CIND or depression risk in both multiplicative and additive (i.e. RERI) scales. Both multiplicative (p-value, 0.02) and additive (RERI, 0.63 [95% CI, 0.13-1.13]) interactions were found between PA and CRP on depression; we observed marginal multiplicative interaction between PA and TNF- α on dementia/CIND (p-value, 0.08), but no additive interaction was observed (RERI, -0.93 [95% CI, -2.10-0.24]).

4.5 Discussion

We found that physical activity prevents death, dementia/CIND, and depression in a cohort of elderly Latino adults corroborating previous studies that linked physical activity to these outcomes.[16, 130] Our study is the first investigation confirming such a link in older Mexican Americans, yet our results from lagged analyses – excluding all cases that occurred in the first 2 years of follow-up - suggested that the PA may affect depression onset in the short term. Importantly, employing a number of biomarkers, we newly found that some of the PA effects on mortality and dementia/CIND and to a lesser extent -if at all - for depression might be mediated through well-known inflammatory pathways represented by biomarkers such as IL-6 and/or TNF-α. However, a large proportion of the PA effects on mortality, cognition, and mood remained unexplained and seem to be operating independently of the inflammatory pathways or the biomarkers we employed.

Our findings of IL-6 and/or TNF- α acting as mediators of the PA effects on mortality and cognition are supported by prior research in humans as well as by animal models. Several elderly cohorts have shown an inverse association between physical activity levels and plasma levels of inflammatory markers,[43] and lower levels of CRP and IL-6 were also observed at follow up among those who were consistently active or reported an increased activity level throughout the study period.[119] Higher levels of inflammation are also associated with chronic medical conditions, including dementia and AD, and are strong predictors of all-cause mortality risk independent of pre-existing morbidity. [44, 113, 115, 131] The anti-inflammatory effects produced by regular physical activity are related to increased production and release of IL-6 and other cytokines by skeletal muscles. [45, 98] Plasma IL-6 increases remarkably during skeletal muscle contraction but declines in the postexercise period; the transient increase in IL-6 levels subsequently triggers the anti-

inflammatory cytokines IL-10 and IL-1 receptor antagonists and suppresses TNF- α levels thus reduce inflammation. [45, 98] Sedentary and inactive lifestyles, on the other hand, cause visceral fat accumulation, and thus activate the inflammatory pathway.[45] In addition, animal studies provided support for exercise-induced neuroimmune cytokine changes in the brain; for example, they reported that hippocampal IL-18 correlated positively with new neuron number in aging rats, in a mouse model of AD exercise reduced brain IL-1β and in rats brain inflammation was reduced after ischemia.[24, 132] Overall, regular exercise results in a reduction of pro-inflammatory cytokines and systemic inflammation and thus presents a potential mechanism for protection against cognitive decline and earlier mortality.

The English Longitudinal Study of Ageing (ELSA) is the only study to date that previously examined and reported that inflammatory markers mediate the association between sedentary behavior and mortality.[133] They found that inflammatory markers (CRP and fibrinogen in log scale) together explained about 16% of the association between sedentary behavior (hours of TV viewing) and mortality, adjusted for physical activity; while dose-response associations between PA and survival was reported in their another publication, mediating effects of inflammation were not examined.[134] In our population, we found that about 10% of the PA and mortality association was mediated by dichotomized IL-6 or TNF- α, but not by dichotomized CRP. Our findings are also in accordance with a one-year moderate PA intervention trial, in which the intervention group of elderly showed reduced systemic concentrations of IL-6 but not CRP compared with the control group that only received health education.[135] Given that CRP is considered a downstream biomarker produced primarily by the liver in response to IL-6 and other inflammatory cytokines, [45, 98] it is not surprising IL-6, stimulated by muscle contraction during physical activity is more specific than CRP in reflecting the mediating effects of inflammation. Additionally, the link

between CRP levels and chronic outcomes may vary according to ethnicity. Though higher CRP levels were observed in Hispanic as compared to non-Hispanic whites,[136, 137] previous studies have shown that CRP appears not to predict cardiovascular disease events among Hispanics or be related to Mild Cognitive Impairment (MCI) among Mexican Americans.[136, 138]

Depression is a common psychiatric disorder in older US adults,[139] and compared with Whites, Mexican Americans, especially immigrants, are more likely to report depressive symptoms and have higher odds of recurrent depression but are less likely to receive standard therapy for depression.[140, 141] We present for the first time an association between PA and depression in older Mexican American and our findings are in line with prior studies in other ethnicities.[130] This suggests that from a health promotion perspective, increasing PA levels even late in life could be an important strategy to prevent depression. Our mediation analyses, however, did not show that any of the biomarkers we employed (CRP, IL-6 and TNF-α) mediated the association of PA and depression, which is consistent with results from a prior study that used a standard approach of simply adjusting for the CRP in regression models. Instead, we, observed both multiplicative and additive interactions between PA and CRP on depression (supplementary table 5), such that the highest risk was estimated for depression for participants with low baseline PA and a high CRP level (HR 1.58, 95%CI 1.17-2.12). Some studies suggested that the positive impact of PA on depressive symptoms may be mediated through psychological mechanisms such as exercise-induced pleasant feelings, and improved self-esteem and self-efficacy,[142, 143] which may explain why inflammation is not on the causal pathways linking PA with depression. In addition, inserting a time lag of two-year with the exclusion of 129 incident depression cases had a substantial effect on our results and became no association between PA and depression, while the positive association remained in 1-year lagged results (excluded 36 depressions). The lagged results suggested a

possible short-term effect of PA, however, the possibility of reverse causation cannot be fully excluded.

The mediation results should be interpreted with some caution. Physical activity level was measured at the same time as the inflammatory markers, and thus the temporal relationship is unclear. Based on the existing literature, however, it is reasonable to hypothesize that a sedentary and inactive lifestyle increases the plasma levels of these biomarkers.[43] In auxiliary analyses, we assessed associations (1) between PA and inflammatory markers, and (2) between inflammation markers and mortality, dementia/CIND or depression risk. As seen in Supplementary table 3 and 4, we found PA to be negatively associated with all markers, and IL-6, TNF- α and TNF- α receptors to be associated with mortality and dementia/CIND, supporting our main mediation results – IL-6 and TNF- α partly mediate the protective PA effects on mortality and dementia/CIND. Finally, similar to other observational studies, we have to assume no unmeasured confounding and while we were able to control for major confounders, residual confounding is always a possibility.

The main strengths of our study are its longitudinal design, our representative sample of the Mexican Americans in California, and our long-term follow up with up to 7 home visits and interviews and nearly 18 years of mortality follow-up. We are the first study that used causal mediation analysis to examine the possible mediating role of plasma inflammatory markers for PA and dementia/CIND, depression or mortality. To examine the role of inflammation, we only include people who had a blood sample and at least one inflammatory marker data in current analyses, yet we found no differences in demographic and baseline health variables between the whole SALSA population and the current subset (Supplementary table 2). Mexican Americans are one of the fastest growing segments of elderly in the U.S. but relatively few studies have investigated risk factors for dementia and depression in this population. Our results suggested that

late-life PA, a modifiable lifestyle factor, protects against the risk of dementia and depression in this population that generally has less adequate access to medical care.[141] The calculated cumulative PA scores (MET-hour/week) in the current study accounted for the intensity and duration of PA and we dichotomized with a cutoff of 35 MET-hour/week in lieu of the IOM guideline. Due to the lack of repeated PA assessments in our cohort, we cannot examine and adjust for the PA changes over time. This may have caused exposure misclassification for those who changed their activity level during follow-up, yet given the prospective design, misclassification of the PA level is nondifferential and this would be most likely bias our results toward the null.

In conclusion, our study results do not only confirm findings from previous studies that being active even at old age protects against cognitive decline, all-cause mortality, and possibly in the short term also for depression, but also suggests that plasma inflammatory markers (especially IL-6 and TNF-α) partly explain the protective PA effects on dementia/CIND and mortality. While further research is required to replicate and understand the mechanism of the PA effects in aging populations, we present the first evidence that anti-inflammation may play a role in cognition and depression among older Mexican American.

4.6 Figure and Tables

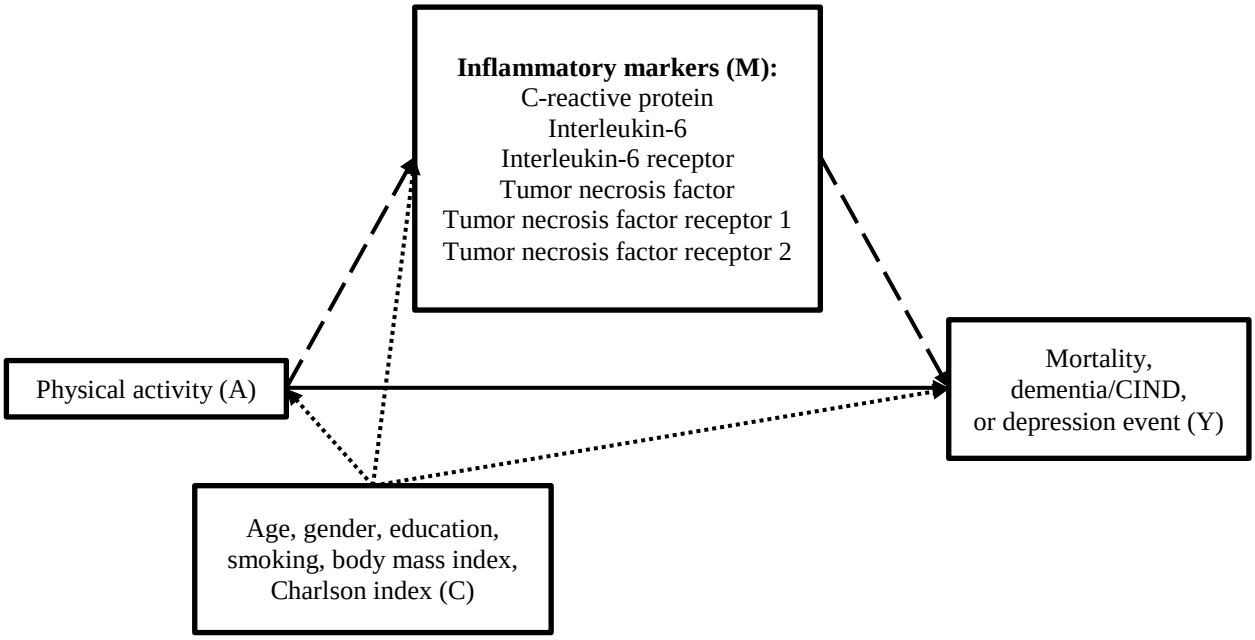


Figure 4-1. Directed acyclic graph

Causal structure of the relations among genetic risk of physical activity (exposure; A), inflammatory markers (mediators; M), and mortality, incidence of dementia/ cognitive impairment without dementia (CIND) or depression (outcome; Y) with measured confounders (C); inflammatory markers are intermediate variables on the causal pathway between exposure (physical activity) and outcomes (mortality, dementia/CIND or depression incidence). The direct effect is represented by the solid arrow and indirect effects are represented by dashed arrows; confounder pathways are depicted as dotted arrows.

Table 4-1. Baseline characteristics of the study participants stratified by physical activity level, Sacramento Area Latino Study on Aging, 1998-2008

	Total (N=1465)	Physical activity [a]	
Variable		High (N=912)	Low (N=553)
Age	70.1 (6.6)	69.8 (6.2)	70.7 (7.1)
Male, n (%)	607 (41.4)	424 (46.5)	183 (33.1)
Education, year	7.5 (5.4)	7.8 (5.4)	7.2 (5.3)
Body Mass Index	29.8 (5.9)	29.5 (5.3)	30.2 (6.8)
Charlson index	0.9 (1.2)	0.8 (1.1)	1.1 (1.3)
Diabetes, n (%)	479 (32.7)	275 (30.2)	204 (36.9)
Stroke, n (%)	127 (8.7)	63 (6.9)	64 (11.6)
Cadiovascular disease, n (%)	528 (36.0)	304 (33.3)	224 (40.5)
Smoking status, n (%)			
Never	683 (46.6)	412 (45.2)	271 (49.0)
Former	615 (42.0)	396 (43.4)	219 (39.6)
Current	167 (11.4)	104 (11.4)	63 (11.4)
***APOE* ε4 carrier, n (%)**	207 (14.5)	135 (15.2)	72 (13.3)
3MSE	86.1 (10.8)	87 (9.8)	84.8 (12.1)
CESD	9.9 (10.6)	8.8 (9.5)	11.8 (12)
Inflammatory Level, Median (mean)			
CRP, mg/L,	3.3 (1.3-7.0)	3.0 (1.2-6.5)	4.2 (1.5-8.4)
IL-6, pg/mL	3.8 (2.5-5.7)	3.5 (2.4-5.3)	4.2 (2.8-6.4)
IL-6 receptor, pg/mL	35538 (27953-43801)	34839 (27441-42948)	36426 (29211-44703)
TNF-α	3.8 (2.9-4.8)	3.7 (2.8-4.6)	3.9 (3.1-5.1)
TNF-α receptor 1, pg/mL	1508 (1284-1843)	1476 (1269-1758)	1603 (1327-1988)
TNF-α receptor 2, pg/mL	2392 (1978-2942)	2320 (1957-2809)	2542 (2078-3227)

APOE, apolipoprotein E; 3MSE, Mini-Mental State Examination; CESD, Center for Epidemiologic Studies Depression Scale; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α.
[a] Physical activity cutoff: 35 MET-hour/week

Table 4-2. Cox Proportional Hazards regression for the association between physical activity and mortality, the risk of dementia/CIND or depression.

Outcome	Event/N	Model 1 HR (95%CI)	Model 2 HR (95%CI)
Mortality	489/1459	1.46 (1.22-1.75)	1.40 (1.17-1.68)
Dementia/CIND	129/1397	1.45 (1.02-2.06)	1.37 (0.96-1.96)
Depression	398/935	1.23 (1.00-1.51)	1.23 (1.00-1.52)

Model 1: adjusted for age, gender, education.
Model 2: adjusted for age, gender, education, smoking, body mass index and Charlson index.

Physical activity exposure was defined as < 35 MET-hour/week (low) versus ≥ 35 (high) MET-hour/week
CIND, cognitive impairment without dementia; CI, confidence interval.

Table 4-3. Mediation analysis on additive hazards model: Direct effects (DE), and indirect effects (IE), of PA level on mortality, risk of dementia/CIND or depression for each inflammatory risk factor, basic adjustment

Effects	Mortality		Dementia/CIND		Depression	
	Additional incident case / 100 000 person-year (95%CI)	Proportion Mediated (95%CI)	Additional incident case / 100 000 person-year (95%CI)	Proportion Mediated (95%CI)	Additional incident case / 100 000 person-year (95%CI)	Proportion Mediated (95%CI)
Potential mediators						
CRP						
DE	1793 (917 to 2668)		643 (104 to 1182)		1696 (-186 to 3579)	
IE	-25 (-108 to 59)	-1% (-7 to 4%)	-22 (-67 to 22)	-4% (-24 to 5%)	50 (-62 to 162)	3% (-12 to 26%)
IL-6						
DE	1580 (710 to 2449)		559 (16 to 1102)		1660 (-231 to 3551)	
IE	157 (50 to 264)	9% (3 to 21%)	69 (10 to 129)	11% (1 to 56%)	136 (-35 to 308)	8% (-14 to 53%)
IL-6 receptor						
DE	1743 (868 to 2619)		633 (111 to 1155)		1838 (-71 to 3747)	
IE	21 (-13 to 54)	1% (-1 to 4%)	-2 (-20 to 15)	0% (-6 to 4%)	-25 (-86 to 36)	-1% (-13 to 6%)
TNF-α						
DE	1535 (659 to 2410)		434 (-108 to 976)		1942 (3 to 3881)	
IE	158 (64 to 253)	9% (3 to 22%)	22 (-29 to 72)	5% (-33 to 60%)	-15 (-195 to 165)	-1% (-24 to 18%)
TNF-α receptor 1						
DE	1524 (655 to 2393)		386 (-164 to 936)		1664 (-263 to 3590)	
IE	177 (49 to 305)	10% (3 to 25%)	84 (12 to 155)	18% (-82 to 137%)	116 (-26 to 258)	7% (-21 to 48%)
TNF-α receptor 2						
DE	1445 (579 to 2312)		354 (-184 to 893)		1713 (-200 to 3626)	
IE	204 (74 to 334)	12% (4 to 30%)	70 (0 to 140)	17% (-100 to 148%)	129 (-65 to 322)	7% (-14 to 53%)

Physical activity exposure was defined as < 35 MET-hour/week (low) versus ≥ 35 (high) MET-hour/week; Baseline inflammation exposure was defined as < Q3 level versus ≥ Q3 level; proportion mediated: the ratio of indirect effect to total effect for low versus high PA level.

CIND, cognitive impairment without dementia; CI, confidence interval; DE, direct effect; IE, indirect effect; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α; DE, direct effect; IE, indirect effect

Model adjusted for Age, gender and education.

Table 4-4. Mediation analysis on additive hazards model: Direct effects (DE), and indirect effects (IE), of PA level on mortality, risk of dementia/CIND or depression for each inflammatory risk factor, full adjustment

	Mortality		Dementia/CIND		Depression	
Effects	Additional incident case / 100 000 person-year (95%CI)	Proportion Mediated (95%CI)	Additional incident case / 100 000 person-year (95%CI)	Proportion Mediated (95%CI)	Additional incident case / 100 000 person-year (95%CI)	Proportion Mediated (95%CI)
Potential mediators						
CRP						
DE	1570 (666 to 2474)		573 (31 to 1115)		1694 (-179 to 3567)	
IE	-49 (-114 to 15)	-3% (-11 to 1%)	-23 (-56 to 9)	-4% (-32 to 5%)	6 (-93 to 105)	0% (-14 to 16%)
IL-6						
DE	1391 (511 to 2271)		512 (-34 to 1058)		1708 (-211 to 3627)	
IE	97 (15 to 180)	7% (1 to 19%)	49 (5 to 94)	9% (-2 to 57%)	51 (-104 to 205)	3% (-16 to 30%)
IL-6 receptor						
DE	1526 (635 to 2416)		565 (24 to 1106)		1833 (-88 to 3755)	
IE	3 (-21 to 27)	0% (-2 to 2%)	-2 (-14 to 9)	0% (-5 to 3%)	-35 (-88 to 19)	-2% (-14 to 5%)
TNF-α						
DE	1383 (502 to 2264)		379 (-170 to 929)		1987 (40 to 3935)	
IE	100 (27 to 173)	7% (2 to 20%)	9 (-32 to 49)	2% (-37 to 48%)	-43 (-210 to 124)	-2% (-23 to 14%)
TNF-α receptor 1						
DE	1400 (508 to 2291)		353 (-192 to 899)		1760 (-113 to 3632)	
IE	98 (4 to 191)	7% (0 to 20%)	50 (-4 to 104)	12% (-80 to 116%)	40 (-78 to 158)	2% (-11 to 22%)
TNF-α receptor 2						
DE	1324 (433 to 2215)		315 (-219 to 849)		1805 (-116 to 3725)	
IE	118 (23 to 212)	8% (1 to 25%)	40 (-13 to 93)	11% (-95 to 129%)	53 (-117 to 223)	3% (-17 to 29%)

Physical activity exposure was defined as < 35 MET-hour/week (low) versus ≥ 35 (high) MET-hour/week; Baseline inflammation exposure was defined as < Q3 level versus ≥ Q3 level; proportion mediated: the ratio of indirect effect to total effect for low versus high PA level.

CIND=cognitive impairment without dementia; CI, confidence interval; DE, direct effect; IE, indirect effect; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α; DE, direct effect; IE, indirect effect

Model adjusted for Age, gender, education, smoking, body mass index and Charlson index.

4.7 Supplement

Supplementary Table 4-S1. Cox Proportional Hazards regression for the association between physical activity and mortality, the risk of dementia/CIND or depression, lagged analyses.

Outcome	Lag 1-year [a]	Lag 2 -year [b]
	HR (95%CI)	HR (95%CI)
Mortality	1.39 (1.16-1.67)	1.37 (1.13-1.65)
Dementia/CIND	1.42 (0.99-2.03)	1.39 (0.95-2.03)
Depression	1.22 (0.98-1.52)	1.15 (0.89-1.49)

Physical activity exposure was defined as < 35 MET-hour/week (low) versus ≥ 35 (high) MET-hour/week

CIND, cognitive impairment without dementia; CI, confidence interval.

Adjusted for age, gender, education, smoking, body mass index and Charlson index.

[a] Lag 1-year: removed 11 deaths, 2 dementia/CIND cases and 36 depression cases in the first year of follow up.
[b] Lag 2-year: removed 46 deaths, 17 dementia/CIND cases and 129 depression cases in the first two years of follow up.

Supplementary Table 4-S2. Baseline characteristics of the study participants with PA data and at least 1 follow-up, and the subset with at least 1 inflammatory marker

Variable	Follow-up participants with PA data (N=1594)	Subset, at least 1 inflammatory marker (N=1465)
Age	70.2 (6.7)	70.1 (6.6)
Male, n (%)	671 (42.1)	607 (41.4)
Education, year	7.4 (5.4)	7.5 (5.4)
Body Mass Index	29.8 (5.9)	29.8 (5.9)
Charlson index	0.9 (1.2)	0.9 (1.2)
Diabetes, n (%)	5151 (32.3)	479 (32.7)
Stroke, n (%)	135 (8.5)	127 (8.7)
Cadiovascular disease, n (%)	580 (36.4)	528 (36.0)
Smoking status, n (%)		
Never	738 (46.3)	683 (46.6)
Former	676 (42.4)	615 (42.0)
Current	180 (11.3)	167 (11.4)
High Physical Activity, n (%)	985 (61.8)	912 (62.3)
3MSE	86.0 (10.9)	86.1 (10.8)
CESD	10.0 (10.5)	9.9 (10.6)

PA, physical activity; 3MSE, Mini-Mental State Examination; CESD, Center for Epidemiologic Studies Depression Scale

[a] Physical activity cutoff: 35 MET-hour/week

Supplementary Table 4-S3. Association between baseline physical activity (exposure) and inflammatory markers (mediators)

	Linear regression [a]			Logistic regression [b]
Mediators	β	SE	p-value	OR (95%CI)
CRP	0.114	0.043	0.008	1.34 (1.06-1.70)
IL-6	0.087	0.028	0.002	1.47 (1.15-1.87)
IL-6 receptor	0.040	0.019	0.036	1.13 (0.88-1.45)
TNF-α	0.043	0.018	0.020	1.42 (1.10-1.84)
TNF-α receptor 1	0.053	0.018	0.003	1.55 (1.19-2.01)
TNF-α receptor 2	0.063	0.018	0.000	1.54 (1.19-1.99)

PA, physical activity; OR, odds ratio; CI, confidence interval; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α.

[a] Linear regression: β represents the difference in biomarker level (log scale) in low PA (< 35 MET-hour/week) versus high PA (≥ 35 MET-hour/week), adjusted for age, gender, education, smoking, body mass index and Charlson index.

[b] Logistic regression: OR represents the risk of high biomarker level in low PA (< 35 MET-hour/week) versus high PA (≥ 35 MET-hour/week), adjusted for age, gender, education, smoking, body mass index and Charlson index.

Supplementary Table 4-S4. Association between baseline levels of inflammatory markers (mediators) on mortality, risk of dementia/CIND or depression (outcomes)

Mediators	Mortality HR (95%CI)	Dementia/CIND HR (95%CI)	Depression HR (95%CI)
CRP	1.05 (0.86-1.28)	0.82 (0.55-1.22)	1.14 (0.92-1.42)
IL-6	1.59 (1.32-1.92)	1.63 (1.13-2.35)	1.18 (0.95-1.47)
IL-6 receptor	1.52 (1.25-1.84)	1.01 (0.67-1.52)	1.02 (0.80-1.31)
TNF-α	1.50 (1.23-1.83)	1.13 (0.76-1.69)	0.98 (0.76-1.27)
TNF-α receptor 1	1.42 (1.16-1.74)	1.59 (1.08-2.36)	1.22 (0.95-1.56)
TNF-α receptor 2	1.49 (1.22-1.82)	1.50 (1.01-2.23)	1.17 (0.91-1.49)

HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α.

[a] the risk of death, dementia/CIND or depression in high level versus low level of biomarker, adjusted for age, gender, education, smoking, body mass index and Charlson index.

Supplementary Table 4-S5. Physical activity (PA) -- inflammatory markers interactions to mortality, risk of dementia/CIND or depression

PA – biomarkers interaction	Mortality				Dementia/CIND				Depression			
	Multiplicative interaction		RERI		Multiplicative interaction		RERI		Multiplicative interaction		RERI	
	coefficient	p-value	HR	95% CI	coefficient	p-value	HR	95% CI	coefficient	p-value	HR	95% CI
CRP	0.05	0.81	0.06	-0.40-0.53	0.07	0.86	-0.01	-0.81-0.79	0.51	0.02	0.63	0.13-1.13
IL-6	-0.15	0.42	-0.05	-0.63-0.54	-0.13	0.73	0.004	-1.17-1.18	0.01	0.95	0.05	-0.50-0.60
IL-6 receptor	0.01	0.98	0.20	-0.44-0.83	0.01	0.97	0.03	-1.00-1.06	0.10	0.70	0.12	-0.46-0.70
TNF-α	0.16	0.42	0.41	-0.17-0.99	-0.72	0.08	-0.93	-2.10-0.24	-0.37	0.16	-0.43	-1.03-0.16
TNF-α receptor 1	-0.02	0.25	0.09	-0.45-0.64	0.07	0.86	0.24	-0.88-1.35	0.13	0.59	0.22	-0.40-0.83
TNF-α receptor 2	0.23	0.25	0.46	-0.07-0.99	-0.06	0.89	0.03	-1.10-1.15	-0.22	0.38	-0.24	-0.87-0.38

CIND, cognitive impairment without dementia; RERI, relative excess risk due to interaction; HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α.

[a] adjusted for age, gender, education, smoking, body mass index and Charlson index.

Chapter 5. Public Health Relevance

Given that the baby boomer population continues to age, it is desirable to identify modifiable risk factors, as well as to develop strategies that could prevent age-related neurodegeneration, and maintain brain health and cognitive performance in older adults. This dissertation examines the influence of physical activity, a modifiable lifestyle, on prevention of PD and cognitive decline and whether the exercise-induced anti-inflammatory effects mediate the preventive effect of physical activity on brain function. We found higher lifetime moderate to vigorous activity and sports activities in youth were inversely associated with PD risk, but no beneficial role for occupational physical activity. Though we cannot exclude the possibility that high PA in the young adulthood is a marker for biologic or genetic factors that lower PD risk, our results provide further support for a previous meta-analysis of 5 studies that concluded higher leisure-time physical activity levels protect against the risk of PD.

Physical exercises are beneficial in terms of many health aspects and have been considered as a recommended non-pharmacological intervention to regulate pro-inflammatory cytokines and anti-inflammatory pathways in reducing atherosclerotic risk factors. Our finding of a large (nearly 10-fold) joint effects of low PA, *APOE* ε4 allele and diabetes on the risk of dementia/CIND provided epidemiologic evidence for PA as an effective preventive strategy against cognitive decline, especially Mexican Americans who are *APOE* ε4 allele carriers with diabetes. In addition, we provide evidence that some of the PA effects on mortality and dementia/CIND might be mediated through well-known inflammatory markers. Such an understanding help identify a high-risk population in Mexican American, a rapidly growing elderly population with a very high rate

of diabetes. Moreover, considering the side effects and the high cost of pharmacological therapies, promote and implement active lifestyle into the aging population, may be an effective, low-risk, and low-cost method that could not only prevent or delay brain degeneration and but also improve older adults' quality of life.

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