ABSTRACT

Title of thesis THE RELATIONSHIP BETWEEN DIETARY INTAKE AND BIOMARKERS OF CAROTENOIDS AND PHYSICAL FUNCTIONING AMONG U.S. OLDER ADULTS

Olfat B. Sheikomar
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Directed By: Professor Nadine Sahyoun, Ph.D., R.D.
Department of Nutrition and Food Science

Decline of physical function [PF] in old age might be related to oxidative damage caused by free radicals, and antioxidants may play a role in reducing the risk of physical functional limitations [PFL]. Yet little is known about the role of carotenoids in PFL. The purpose of this study was to assess the association of total and daily dietary intakes of carotenoids, fruit and vegetables [FV] and their biomarkers with PF among U.S. older adults. Data were from 2,905 men and women [≥ 60 years] in the National Health and Nutrition Examination Survey [NHANES] 2003-2006. Using logistic regression, we found that serum concentration of carotenoids was associated with limitations in PF. In the fully adjusted model, the ORs [95% CI] of having limitation in activities of daily living [ADLs], instrumental activities of daily living [IADLs] and movement difficulties [MD] were 2.03 [1.16 - 3.53], 2.34 [1.61 - 3.42], and 2.15 [1.46 - 3.18], respectively, comparing the lowest quintile of serum carotenoids to the highest. Total intake and dietary intake of carotenoids were found to be associated with limitations in IADL. However, low FV consumptions were not significantly associated with PF domains. In conclusion, elevated levels of serum carotenoids are significantly associated with better physical functional performance and may play an important role in delaying the onset of physical decline.

THE RELATIONSHIP BETWEEN DIETARY INTAKE AND BIOMARKERS OF CARTENOIDS AND PHYSICAL FUNCTIONING AMOUNT U.S. OLDER ADULTS

By
Olfat Bakur Sheikomar

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Advisory Committee:
Dr. Nadine Sahyoun, Advisor and Chair
Dr. Hee-Jung Song
Dr. Mira Mehta

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Chapter 1: Introduction

In the United States [U.S.], the population1 of older adults has been rapidly increasing. Aging is associated with the accumulation of free radicals leading to oxidative stress, which is highly associated with deterioration of physical function [PF], caused by damage to muscle tissue (Barbara Marzani, 2004; Fano et al., 2001; Semba, Ferrucci, et al., 2007). To protect against this oxidative stress and to reduce damage to muscle tissues, it has been suggested that carotenoids may penetrate and get stored in intramuscular fat working closely to protect muscle tissue (Bohm, Edge, & Truscott, 2012; Lobo, Patil, Phatak, & Chandra, 2010; Semba, Lauretani, & Ferrucci, 2007). Essentially, carotenoids, including α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene, are fat-soluble antioxidants which are abundantly available in fruits and vegetables. Through this mechanism, carotenoids may prevent or at least delay the onset and complications of physical functional limitation [PFL]. Therefore, to prevent PFL, it is important to understand its association with dietary intake and plasma concentrations of carotenoids. More importantly, the results of this study may contribute to the development of effective strategies and interventions, aimed at preventing progression to disability and improving disability management among older adults. Such preventive activities may lead to higher levels of functioning for older adults and help retain the ability to live independently.

1.1 Background

The latest data show that there is a steady increase in the U.S. older adult population, and it will continue to grow (M. W. Brault, 2012). It is predicted that by 2030 the number of U.S. adults aged 65 and older will be approximately 71 million, more than twice the number in 2000, representing 19.3% of the U.S. population (AoA, 2010; M. Brault, Hootman, J., Helmick, C., Theis, K., & Armour, B., 2009).

Multiple factors have contributed to population growth in recent years. Primarily, this growth is due to the aging of the baby boom population, whose members were born primarily

between 1946 and 1964. Additionally, the decline in age-specific mortality and the increase in life expectancy have contributed to the increase in the number of older adults. Life expectancy at birth has increased from 68.6 years in 1950-1955 to 75.8 years in 1990-1995, and it is expected to reach 79.1 years by 2015 (Joseph & Suzman, 2010; United Nations, 2013). Life expectancy at both age 65 and age 85 has also increased. People who survive to age 65 can expect to live for an average of 19.2 more years, and people who survive to age 85 can expect to live for an average of 7 more years for women and 5.9 more years for men (FIFARS, 2012). Particularly, men and women at 65 years can expect to live for an additional 17.7 years and 20.3 years, respectively (Murphy, Xu, & Kochanek, 2013). The availability of health care resources and greater socioeconomic advantages are more likely to increase life expectancy. All of these factors simultaneously are playing a crucial role in the growth of the older population.

This population trend has huge implications for older adult support programs and medical services, as well as future social, economic and health concerns associated with aging (Meydani, 2002). One of these concerns is the decline in PF, which is related to an individual's ability to perform activities required in the daily lives (Painter, Stewart, & Carey, 1999). As the population aged, there has been an increase in the number of people with PFL. The data from the 2001–2007 *National Health Interview Survey* [NHIS] show that the prevalence of difficulties in doing certain activities [e.g. walk a quarter of a mile; walk up 10 steps without resting; stand or be on your feet for about two hours; sit for about two hours; stoop, bend, or kneel; reach up over your head; use your fingers to grasp or handle small objects; lift or carry something as heavy as 10 pounds], among adults ages 50-59, 60-69, 70-79, and 80 and older who have one or more PFL, is 16.5%, 22.9%, 31.4%, and 42.9%, respectively. The presence of one or more physical limitations among older adults, aged

80 years and over, is 2.5 times as likely as in adults aged 50-59 (Holmes, Powell-Griner, & Lethbridge-Cejku, 2009). Thus, as a person advances in age, the risk of developing PFL increases.

A "Summary Health Statistics for U.S. Adults" report issued by the NHIS in 2012 showed that in the U.S. about 40 million persons aged 65 years and older [12%] were limited in their usual physical functioning due to one or more chronic health conditions (Schiller JS, 2012). Particularly, among adults aged 65 years and older, 23.7 million [62%] had impairment in at least one of the activities of daily living [ADL: i.e. ability to eat, dress and transfer] or instrumental activities of daily living [IADL: i.e. managing money, preparing meal and doing chores] (Murphy et al., 2013). Moreover, 3.3% of adults aged 65 to74 needed help with ADL from another person, and 11% of adults aged 75 and older required the help of another person with ADL, and 20% required help with IADL (Murphy et al., 2013). So, the absolute number of older adults with PFL is increasing among the U.S. older population.

PFLs are adversely associated with the ability to live independently and with the overall quality of life. Thus, older adults who need help with ADL and IADL are at higher risk of losing the ability to live independently in the community and often need care at home (Kramarow, Lubitz, Lentzner, & Gorina, 2007). Moreover, a decline in PF is typically accompanied by a subsequent deterioration in dietary quality and a compromised nutritional status (Meydani, 2002). This may be due to barriers in accessing fresh fruits and vegetables [FV] and preparing and consuming these nutrient-rich foods (Sahyoun, Zhang, & Serdula, 2005).

Several studies have indicated that older adults tend to eat more FV compared to other age groups. However, the consumed amounts are still less than those recommended by the Dietary Guidelines for each age-gender group. Based on data from *National Health and Nutrition Examination Survey* [NHANES] 1999-2002, 72% and 95% of Americans who are 19 years and

older consume at least one serving of fruit or vegetables on a given day, respectively. Compared with younger adults[< 60 years], older adults [≥ 60 years] tend to eat significantly more fruit on a given day than their younger counterparts, but there were no significant differences related to vegetable consumption (Juan & Lino, 2007). However, only about 21% to 37% of men and 29% to 45% of women aged 65 years and older achieve the recommended five servings of FV per day as estimated by major studies (Li et al., 2000; Nicklett & Kadell, 2013).

Observational studies have found an inverse association between antioxidants or FV intake and PFL, walking speed and muscle strength (Alipanah et al., 2009; Cesari, Penninx, et al., 2004; Denise K Houston, Stevens, Cai, & Haines, 2005; Lauretani et al., 2008; Myint et al., 2007; S. M. Robinson et al., 2008; Tomey et al., 2008). FV are the primary dietary sources of carotenoids, including α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene. Carotenoids are responsible for the yellow, orange and red color of the FV in varying concentrations. Also, a systematic review identified carotenoids as the best serum biomarkers of FV consumptions (Baldrick, Woodside, Elborn, Young, & McKinley, 2011). Thus, the higher the FV intake, the higher the carotenoid levels in the serum.

The exact mechanism through which FV may impact PF and muscle strength is not entirely understood. But, it is suggested that with advanced age the excessive oxidative damage increases. Oxidative damage perpetrated by reactive oxygen species [ROS] causes a disruption in the equilibrium of the biological system by damaging its major constituent molecules, such as protein, lipids and deoxyribonucleic acid [DNA], leading to cell death (Lobo et al., 2010).This in turn leads to a decrease in muscle mass and strength and decline in PF (De la Fuente, 2002; Fano et al., 2001; Lauretani et al., 2008; Semba, Lauretani, et al., 2007). High content of carotenoids in FV may protect against the accumulation of oxidative stress and prevent its damage (Cesari, Penninx, et al., 2004; Neville et al., 2013). The double bond system of the carotenoid results in a high capacity for quenching single oxygen and efficiently acting as

free radical scavengers, inhibiting lipid peroxidation and protecting DNA against oxidative damage (Stahl & Sies, 2005). Furthermore, since carotenoids are fat-soluble antioxidants, they may penetrate intramuscular fat, preventing or reducing the process of oxidative stress within the muscle tissues. Thus, it may avert damage in muscles cells and maintain mass and strength, which may eventually lead to reducing the risk and delaying the onset or the complications of PFL.

Based on the literature, there is a positive association between antioxidants [e.g. vitamin C, vitamin E, retinol and only dietary intake of β-carotene or serum level of carotenoids] and PFL or muscle strength. However, there is limited information on the association between PFL and both dietary intake estimate of carotenoids and their biomarkers among a representative sample of U.S. older adults. This study will examine those associations and use the PFL classification suggested by *National Center for Health Statistics* [NCHS]. Also, muscle mass percentage and dietary protein intake is available and will be controlled for in this analysis.

There is a consensus conclusion that skeletal muscle loss leads to PFL, which may affect the quality of life and increase the need for supportive services among older adults. Studies aimed at establishing whether FV intakes and dietary intakes of carotenoids and their biomarkers are related to PFL among U.S. adults, aged 60 years and older, using NHANES 2003-2006 is of interest for potential intervention in the prevention of muscle loss.

Chapter 2: Literature Review

2.1 Overview of PF

One of the major health concerns facing older adults is a decline in physical functional performance. Physical functioning is best defined as the ability to perform activities required in daily living. (Painter et al., 1999). The level of physical performance is a reflection of an individual's overall health and is linked to: 1) lower quality of life and loss of independence; 2) higher risk of disabilities, falls and fractures; and 3) higher health care costs (Painter, 2005).

2.2 Assessing PF

The assessment of PF among older adults includes measuring strength, mobility, freedom of movement, and balance and coordination (CDC, 1993). These functions are assessed through either self-reporting or standardized performance measures.

2.2.1 Self-reported PF

There are several questions that measure PF related to three types of functioning: 1) activities of daily living [ADLs]; 2) instrumental activities of daily living [IADLs]; and 3) movement difficulty [MD]. These questions are used in most of the national surveys, including NHANES.

2.2.1.1 Activities of daily living [ADLs]

An assessment of ADLs can gauge an individual's ability to perform a wide range of daily tasks. In fact, the American Occupational Therapy Foundation and Association and the U.S. National Institutes on Aging developed the "Assessment of Motor and Process Skills," which includes more than 100 standardized ADLs tasks. However, the "Katz Index of Independence in ADLs" includes six activities [i.e., bathing, dressing, toileting, transferring, continence, and feeding].

2.2.1.2 Instrumental activities of daily living [IADLs]

IADLs are used to assess independent living skills that are more complex than the basic ADLs. The capacity to handle these complex functions is lost before the ability to complete basic ADLs. The Lawton-Brody scale is one of the assessment tools for IADLs and assesses the ability to use the telephone, shop, prepare food, do light housekeeping and laundry, handle transportation, take responsibility for medications, and manage money (Graf, 2008).

2.2.1.3 Movement difficulty [MD]

MD includes lower-extremity function [LEF] and upper body function [UBF]. Results of a prospective study of 7,575 women aged 75 years and older with no history of hip fracture showed that women with lower speed gait and difficulty doing tandem walk have higher incidences of falls and fractures [relative risk: 1.4, 95% Cl 1.1-1.6]. This study concluded that LEF is considered a strong predictor of disability and has been associated with falls and hip fractures among older adults (Dargent-Molina et al., 1996). The loss of function generally occurs less in the UBF than in the LEF with aging.

2.2.2 Standardized measurements of function

Objective assessments include standardized tasks such as measuring walking speed, testing balance and grip strength, and sit-to-stand tests, which are used generally in smaller research settings or in a subsample of a large national survey (Lang, 2011).

2.3 Risk factors of impaired PF

PFL is multifactorial and may be attributed to oxidative stress, obesity, physical inactivity, alcohol consumption, smoking, and genetic factors (Semba et al., 2003). Also, chronic diseases and inflammation may be associated with poor strength, incident mobility disability, a decline in walking speed, and poor physical performance (Cesari, Penninx, et al., 2004; Denise K Houston et al., 2005; Semba,

Lauretani, et al., 2007). Malnutrition may play a crucial role in the decline of PF. The association between diet and PFL could be described as vicious cycle: The decline in muscle mass and physical capability may increase the risk of malnutrition and poor quality of life while malnutrition may contribute to further decline in muscle mass and strength leading to PFL and disability.

2.4 Mechanism of ROS, protein, and antioxidants with PF

The causes of aging have not been elucidated. One theory attributes aging to oxidative damage induced by free radicals. In addition, several studies suggest that the pathogenesis of age-related physical dysfunction is multifactorial and it may be attributed to malnutrition and inactivity besides oxidative stress (Semba, Lauretani, et al., 2007). Skeletal muscles are vulnerable to oxidative stress because they use a large amount of oxygen and they are likely to accumulate oxidative damages over time. The damage is extended to macromolecules such as muscle proteins, which subsequently cause a decline in muscle mass and strength leading to PFL (Semba, Lauretani, et al., 2007; Barbara Marzani, 2004). This atrophy in the muscle tissue caused by oxidative stress is associated with a progressive loss of muscle mass and strength, or sarcopenia. An outcome of sarcopenia is disability due to decreased mobility and an increased risk of falls and frailty (Lauretani et al., 2003). There is an increasing interest in the role of oxidative stress in the etiology of sarcopenia, and biomarkers of oxidative stress, such as F2-isoprostane, have been shown to predict PFL (Holt, 2009).

Moreover, as older adults consume smaller amounts of food, inadequate food intake may result in inadequate protein and FV consumptions leading to lower amino acid and antioxidant capability, which may have a greater detrimental effect on muscles mass and the anabolic process (Lauretani et al., 2008; D. Paddon-Jones & Leidy, 2014; Semba, Lauretani, et al., 2007). Based on the literature, antioxidants and protein intakes are promising nutrients in maintaining muscle mass. To counteract sarcopenia and its consequences, nutritional interventions to increase protein and antioxidant consumptions

may play crucial roles. For instance, compelling data support the ability of dietary protein to stimulate muscle protein synthesis in aging individuals, suggesting a need to moderately increase the recommended dietary allowance [RDA] of protein from 0.8 g/kg/day to 1.0-1.5 g/kg/day to promote optimal health and maintain muscle mass and strength among older adults (D. Paddon-Jones & Leidy, 2014; Douglas Paddon-Jones, Short, Campbell, Volpi, & Wolfe, 2008; Mosoni et al., 2014). Likewise, increasing intake of antioxidants, such as carotenoids, vitamin C and vitamin E, plays a vital role in neutralizing free radicals (Lobo et al., 2010) and thus may maintain muscle mass and strength. Thus, dietary intervention through increasing protein and antioxidant consumption simultaneously is considered a simple strategy to maintain muscle mass and strength or even delay the onset of physical functional limitation.

In summary, accumulated ROS may cause damage to cells and tissues, leading to impaired cell function. However, the presence of antioxidants may neutralize the ROS and prevent cell damage, and increasing the protein intake may support muscle anabolic and the regeneration of the damaged muscle tissue, which may prevent or delay PFL.

2.4.1 Free radicals and antioxidants.

Free radicals are produced during metabolism and as an outcome of environmental factors, including smoking, pesticides, pollution, and radiation. Free radicals are defined as unstable molecules, due to a missing electron, which react easily with biomolecules of the human body, such as DNA, lipids and proteins (Mecocci et al., 1999). Consequently, when a free radical attacks a molecule, the molecule becomes a free radical itself, causing a chain of reactions that can destroy cells and tissues. Free radicals may also increase the risk of several diseases such as cancer and cardiovascular disease. Antioxidants are molecules that are capable of neutralizing free radicals by giving up their electrons without becoming free radicals themselves. So, antioxidants prevent

cellular damage and subsequently reduce the risk of diseases and aging (Shu, Cheung, Khor, Chen, & Kong, 2010).There are several forms of antioxidants, including phytonutrients such as carotenoids and vitamins, including vitamin C and vitamin E, that can be found in most plant-based food such as fruits, vegetables, grains and herbs.

2.4.2 Oxidative stress and muscle mass decline

Free radical damage targets the mitochondria, which is considered the center of aerobic metabolism, causing mitochondrial dysfunction (Kim, Wilson, & Lee, 2010; Mecocci et al., 1999). Mitochondrial dysfunction is positively associated with aging, which may lead to a decline in respiratory chain function. Basically, respiratory-chain-deficient cells are prone to apoptosis, which may damage human tissue such as the skeletal muscle. Thus, increased cell loss is a consequence of age-associated mitochondrial dysfunction (Trifunovic & Larsson, 2008). In addition, there is a tendency to link mitochondrial dysfunction to an increased generation of ROS (Wickens, 2001). Accumulated ROS leads to excessive oxidative stress, causes significant damage to cells and tissues, and, in particular, may affect skeletal muscle morphology and function. Aging muscle shows increased damage to biomolecules, which are implicated in the pathogenesis of losing muscle strength and mass (Lauretani et al., 2008; Semba, Lauretani, et al., 2007). In addition, ROS provides a trigger for the expression of inflammatory cytokines such as interleukin [IL]-1β, tumor necrosis factor [TNF]-α, C-reactive protein, and IL-6, which are positively associated with PFL (Cesari, Penninx, et al., 2004; Hozawa et al., 2007; Walston et al., 2006). An additional plausible theory for age-related loss in muscle tissue considers the effect of oxidative stress on muscle satellite cells, which play important roles in regenerating muscle (Morgan & Partridge, 2003). It was shown that ROS induces a loss of viability, a shorter lifespan, and a decrease in the proliferative capacity of satellite cells. It was also demonstrated that the

number of muscle satellite cells decreases with aging, which may cause a reduction in myofiber number and consequently decrease the ability to regenerate the myofiber (Kim et al., 2010).

Based on the free radical theory, several epidemiological studies suggest that oxidative damage perpetrated by free radicals may play a crucial role in the decline of functional activity in human skeletal muscle with normal aging (De la Fuente, 2002; Fano et al., 2001; Barbara Marzani, 2004; Cesari, Pahor, et al., 2004; Semba, Ferrucci, et al., 2007). *The Women's Health and Aging Study [*2007] showed that indicators of oxidative stress were associated with a decrease in walking speed over 36 months of follow-up, which increased the incidence of severe walking disability with a Hazard Ratio [HR] = 1.42, 95% CI, 1.02–1.98, P = 0.04 (Semba, Ferrucci, et al., 2007).

Since ROS increases with aging, an equilibrium between free radical production and antioxidant-linked inactivation is required to preserve health. One theory indicates that functional decline may be the result of an imbalance between free radical production and antioxidant deficits with concomitant oxidative stress. ROS may be neutralized through increasing dietary antioxidant intake. Cohesive evidence shows that most diets that protect against oxidative stress and inflammation are mainly made up of plant-based foods, which are high in phytonutrients and antioxidants (Boeing et al., 2012).

2.4.3 What are phytonutrients?

All plant foods contain a wide range of biologically active compounds that are known as phytonutrients. "Phyto" is a Greek term meaning plant and the word phytonutrients refers to useful plant natural pigments with different structures that determine particular properties in plants and provide much of the flavor and color of edible plants (USDA, 2005). FV are concentrated sources of phytonutrients and tend to have the highest levels. Other plant foods like teas, whole grains,

legumes/beans, nuts, seeds, herbs and spices also contain phytonutrients (Carter, Gray, Troughton, Khunti, & Davies, 2010; NHI, 2011).

2.4.4 Fruit and vegetable consumption reduce oxidative stress

Diets rich in FV, the primary source of phytonutrients such as carotenoids, have been associated with a decreased incidence of inflammation and oxidative stress (Rink et al., 2013). In a cross-sectional study, conducted by Holt et al. in 2009, 285 adolescent boys and girls aged 13 to 17 years were assessed. Results showed that serum C-reactive protein [CRP] was significantly inversely associated with intake of fruit [r = -0.19; P = 0.004]. Specifically, the results show that β-carotene was inversely associated with urinary F2-isoprostane, which is a marker of oxidative stress, serum IL-6 [r = -0.15; P<0.05] and serum TNF-α [r = -0.14, P=0.02]. Overall, this study suggested beneficial effects of high intake of FV on lowering the markers of inflammation and oxidative stress (Holt et al., 2009).

A recent longitudinal study investigated the effect of meeting "five a day" FV consumptions on biomarkers of oxidative damage and antioxidant defense. Study participants, 258 healthy, premenopausal women, were followed for ≤ 2 menstrual cycles with ≤ 16 oxidative stress measures. Linear mixed models with repeated measures showed an inverse association between consuming five FV a day and oxidative stress [24-hour recall β = -0.10 [95% CI, -0.12 to -0.07]; FFQ β = -0.14 [95% CI, -0.18 to -0.11]] (Rink et al., 2013).

2.4.6 Carotenoids and PFL

2.4.6.1 Dietary Carotenoids

One of the major categories of phytochemicals is carotenoids. More than 600 carotenoids have been identified in nature. These fat-soluble pigment compounds are synthesized by plants and microorganisms, but not by animals or humans. Carotenoids are responsible for the yellow,

orange and red colors of FV, in varying concentrations, which constitute the major sources of carotenoids in the human diet. However, only 40 carotenoids are present in a typical human diet, 20 of which are identified in human blood and tissues. Approximately 90% of carotenoids in the diet and the human body are represented by six carotenoids under two classes, namely: xanthophylls [which contain oxygen in their chemical structure such as lutein zeaxanthin and β-cryptoxanthin] and carotenes [which are purely hydrocarbons, and contain no oxygen in their chemical structure] such as α-carotene, β-carotene, and lycopene (NHI, 2011; Rao & Rao, 2007). Carotenoids are an important element of the fat-soluble antioxidants defense system in humans. Carotenoids function by quenching singlet oxygen, scavenging free radicals and inhibiting lipid peroxidation (Rao & Rao, 2007; Semba, Lauretani, et al., 2007). Some of the common food sources of carotenoids that characteristically have bright colors include carrots, broccoli, sweet potatoes, pumpkin, citrus fruits, peaches, apricots, kale, spinach, turnips, tomatoes, pink grapefruit, watermelon, pink guava, butternut squash, red bell peppers and cantaloupes (Buijsse, Feskens, Kwape, Kok, & Kromhout, 2008; NHI, 2011; Santos et al., 1998).

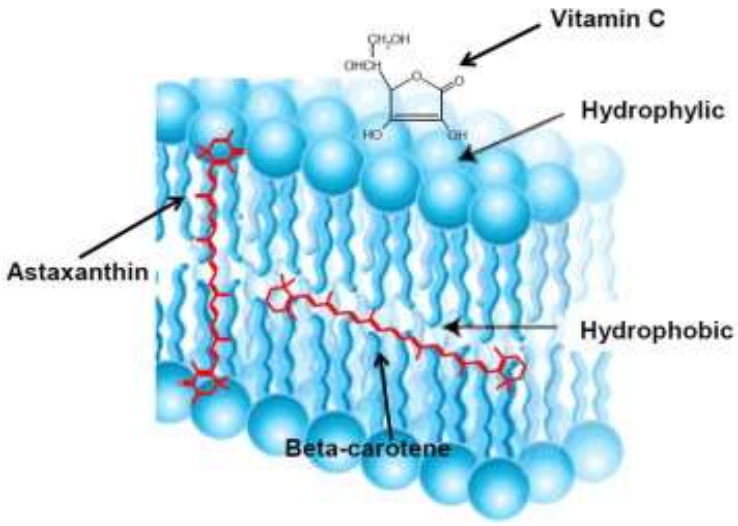


Fig. 1 The localization of carotenoids in biological membranes (Jomova & Valko, 2013).

2.4.6.2 Bioavailability of carotenoids

There is a strong association between FV intake and dietary intake of carotenoids. However, several factors appear to affect the bioavailability of carotenoids or the response of carotenoids to increased FV consumption, including agricultural methods like: harvesting season, production practices, post-harvest handling, processing and storage. In fact, as the maturity stage of FV increase, the carotenoids increase (Schreiner & Huyskens-Keil, 2007; Voutilainen, Nurmi, Mursu, & Rissanen, 2006). Also, the presence of dietary fat enhances the absorption of carotenoids, because they are fat-soluble nutrients. Heated and processed foods contain more carotenoids than unprocessed and raw sources. Most importantly, the health status of an individual could affect the absorption of carotenoids as well as the food matrices (Schreiner & Huyskens-Keil, 2007; Voutilainen et al., 2006).

2.4.6.3 Carotenoids as biomarkers of FV intakes

Six carotenoids [i.e. α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin] are found in significant and appreciable amounts in human serum. Total serum carotenoids have been explored as potentially useful indicators of FV intakes, since they are the primary source of carotenoids in the diet and cannot be synthesized inside human bodies (Baldrick et al., 2011; Woodside, Young, & McKinley, 2013). Moreover, a systematic review has investigated the main biomarkers of FV intakes, such as vitamin C, carotenoids and flavonoids, in whole diet, mixed or individual varieties of FV. The results of this systematic review show that carotenoids and vitamin C are the most frequently measured FV consumption biomarkers (Baldrick et al., 2011; El-Sohemy et al., 2002).

2.4.7 Fruit and vegetable consumptions and PFL

The suggested mechanism through which FV may influence PF and muscle strength may be related to high antioxidant content, thereby, protecting against oxidative stress and inflammation (Cesari, Penninx, et al., 2004; Mecocci et al., 1999). Previously, epidemiological and intervention studies have indicated

that a diet rich in FV has been associated with a lowered risk of a number of age-degenerative diseases, including cardiovascular disease, cancer and diabetes (Ford & Mokdad, 2001; Hu, 2003; Hung et al., 2004; Rolls, Ello-Martin, & Tohill, 2004; Tohill, Seymour, Serdula, Kettel-Khan, & Rolls, 2004). Additionally, several studies report an association between FV intake and/or nutrients associated with a diet high in FV, such as β-carotene, tocopherol, vitamin C, flavonoids, and other polyphenols and enhanced physical function and muscle strength (Cesari, Pahor, et al., 2004; Denise K Houston et al., 2005; Semba et al., 2003). A population-based, cross-sectional study of 16,792 individuals aged 40-79 years in Norfolk, United Kingdom [UK] investigated the association between FV consumptions, collected in a food frequency questionnaire and self-reported physical and mental functional health measured in a short-form, 36-item questionnaire. The mean of the UK SF-36 physical component summary scores was significantly higher with higher total FV consumption in both men and women [P = 0.0001]. Also, men and women with a high consumption of FV were more likely to report good physical health compared to those with lower consumption [odds ratio [OR] 1.30, 95% confidence interval [CI] 1.11–1.53 for men and OR 1.28, 95% CI 1.11–1.48 for women]. In conclusion, higher FV intake is associated with better self-reported physical functional health (Myint et al., 2007). In this study, physical activity [PA] was not controlled for in the model.

In the prospective *Atherosclerosis Risk in Communities* [ARIC] study conducted by Houston et al. [2005], the association between the baseline intakes of FV, estimated using FFQ and a self-reported PF questionnaire administered nine years later, among 9,404 participants aged 45-64 at the baseline time was assessed. The questionnaire included 12 activities that were classified into lower-extremity function [LEF] ADLs, and IADLs. Logistic regression showed an inverse association between baseline FV intakes with LEF, ADLs, and IADLs. This study concluded that among older adults lower FV intakes were related to greater functional limitations

(Denise K Houston et al., 2005). The analysis of the ARIC data controlled for fewer covariates, including age, gender, total energy intake, BMI, education, race/ethnicity, and smoking status, and did not control for PA, self-rate health status, and C-reactive protein.

As part of the longitudinal *Study of Women's Health across the Nation* [SWAN], Tomey et al. [2008], prospectively examined the association between diet quality and number of FV servings with prevalence of physical functioning limitations four years later among 2,160 women aged 42-52. Physical functioning status was assessed using a 10-question subscale of the Medical Outcomes Study Short Form 36, which consisted of questions inquiring about the level of difficulty performing daily tasks such as bathing, dressing, carrying groceries, bending, moderate and vigorous athletic activities, walking, and climbing stairs. Logistic regression showed that lower FV intakes were related to greater functional limitation. Also, this study suggested that participants in the lowest quartile of dietary lycopene, adjusted for total energy intake, were 40% more likely to develop physical limitations than those in the highest quartile (Tomey et al., 2008). The SWAN study included only younger women of different ethnicities excluding NH-white. FV intakes were assessed using FFQ and a PF questionnaire that included only 10 scored questions, which limited the overall assessment of PF status.

The only randomized controlled intervention study "Aging and Dietary Intervention Trial [ADIT]" undertaken by Neville et al. in 2013 examined the effect of increasing FV consumptions on grip strength and LEF. Eighty-three participants, aged 65–85 years, habitually consuming ≤ 2 portions of FV per day, were randomized to maintain intake or to increase intake to ≥ 5 portions of FV for 16 weeks. The result was marginally significant but showed a trend toward an increase in grip strength among the group consuming a higher intake of FV, [mean change at 16 weeks ± SD, 2.04±5.16 and 0.11±3.26 kg, respectively $P = 0.06$] (Neville et al., 2013). The intervention period

may have been too short to detect a stronger effect of FV intake on PFL. Moreover, the PF status was determined in the ADIT study by the Short Physical Performance Battery [SPPB] which is considered an objective test of PF and may be more accurate than the subjective questionnaire, but only tests LEF.

2.4.7.1 Association between carotenoids and PF

Several observational studies have addressed the association between antioxidants, including dietary vitamin C, vitamin E, β-carotene and total serum carotenoids with physical functional performance, using different approaches and study designs (Cesari, Pahor, et al., 2004; Tomey et al., 2008). Yet little is known about the mechanism of dietary and serum carotenoids in preventing functional limitations and disability.

The cross-sectional *Women's Health Aging Study* [WHAS] aimed to assess the association between serum carotenoids and hip, knee and grip strength among 669 women aged 70-79. Results from the WHAS showed that low plasma carotenoids were independently associated with poor skeletal muscle strength and impaired physical performance (Semba et al., 2003).

One of the first studies was based on InCHIANTI data, a nationally representative cross-sectional study of 986 Italians, aged 65 years and older (Cesari, Pahor, et al., 2004). Findings of this study suggested a positive association between plasma α- and γ- tocopherols and muscle strength [β= 0.566; SE=0.193; p = 0.003, β= 0.327; SE=0.165; p = 0.04], respectively. In addition, the results indicated that higher dietary intake of antioxidants, adjusted for total energy intake especially vitamin C and β-carotene, were positively associated with higher skeletal muscular strength in elderly persons [β= 0.383; SE=0.162; p = 0.02, β= 0.311; SE=0.159; p = 0.05, respectively]. This study missed the simultaneous evaluation of the same dietary antioxidant intakes and their serum biomarkers, which might better identify the effects of antioxidants on PF.

Among the same cohort, Laurentani et al. [2008] investigated, prospectively, the potential effect of plasma carotenoids, on hip, knee and grip strength measured at baseline and six years later among 628 men and women. Findings of this study showed that participants with lower plasma concentrations experienced a greater decline in hip, knee and grip muscle strength over a period of six years compared to persons with higher plasma carotenoids (Lauretani et al., 2008). The assessment of PF status was comprehensive in this study; both measures of lower and upper extremity muscle strength were included such as hip, knee and grip strength as well as the SPPB. Although this study was mainly focused on sarcopenia as predicted by muscle strength, it did not control for dietary protein intake, which plays an important role in maintaining muscle mass and strength.

Several studies have investigated the association between carotenoids, including α-carotene, β-carotene, lycopene, lutein zeaxanthin and β-cryptoxanthin, and ADLs, IADLs and MD. However, few studies have simultaneously examined dietary and supplement intake of carotenoids, their biomarkers and FV intake in association with self-reported PFL. Most of the previous studies have assessed PFL by assessing muscle strength. Although assessing muscle strength is an important indicator of functioning and risk of falls, fracture and disability, assessing ADL, IADL and MD may be another approach to measure independent living. In fact, none of the previous studies have tested the association of carotenoids with ADL, IADL and MD. Furthermore, previous studies did not examine the relationship between dietary intake of FV and carotenoids among nationally representative populations in the U.S. using NHANES data.

2.5 Rationale

Functional limitations and impairment due to loss of muscle mass and strength [sarcopenia] reduce the overall quality of life and compromise functional independence. The loss of

independence and the impaired ability to do daily activities such as shopping, preparing a meal, and eating may potentially spiral into malnutrition and poorer health. Furthermore, PFL may lead to an increase in the risk of falls and fractures. Thus, a high incidence of PFL, along with related complications and consequences, may increase the need for institutionalization as well as increase unprecedented demands on the health care system and senior services. Therefore, an understanding of the nutritional factors that may delay or possibly prevent physical dysfunction is critical for improving the quality of life as well as for potentially decreasing the estimated health care expenditures among the aging population. The association between carotenoids and PF must be investigated as part of the many efforts to find ways to improve the overall health of older adults and to help them remain independent.

Previous studies that examined the relationship between PFL, or muscle strength, and serum or dietary antioxidants suggested a potential effect of antioxidants in reducing the risk of physical dysfunction and increasing the muscle strength. However, these studies concentrated on total FV intake, dietary intake of β-carotene or serum concentration of carotenoids. This is the first study that combined total intake and dietary intake of carotenoids, total FV intake and their biomarkers to examine their associations with PF.

Furthermore, previous studies primarily focused on lower-extremities assessment to predict PF status. In NHANES, although the assessment of PF is self-reported, it is comprehensive in assessing functioning of both the upper and lower extremity. Based on the literature, there is limited information about the association between carotenoids and PFL, classified into: ADL; IADL; and MD. Furthermore, the analysis controlled for lean body mass and dietary protein intake. . There are no nationally representative studies among U.S. older men and women that have previously documented this association. Therefore, the purpose of this study is to assess the

association between dietary intakes and plasma concentrations of carotenoids and comprehensive self-reported assessment of physical functioning.

2.6 Research questions

1. What are the characteristics of the people with lower and higher PFL in terms of socio-demographics and lifestyle factors?
2. Is there an association between plasma level of carotenoids and PFL of ADL, IADL and MD among older adults?
3. Is there an association between energy-adjusted, and unadjusted dietary intake of carotenoids and PFL of ADL, IADL and MD among older adults?
4. Is there an association between total intake of carotenoids [diet + supplement] and PFL of ADL, IADL and MD among older adults?
5. Is there an association between total FV intake and PFL of ADL, IADL and MD among older adults?

Chapter 3: Methods

3.1 Database and Study Population

The NHANES is conducted by the National Center for Health Statistics [NCHS] of the Centers for Disease Control and Prevention [CDC]. NHANES is a series of complex cross-sectional surveys that involve multistage and a probability cluster sampling design. Since 1999, the survey collects data on a yearly basis on a sample of 5,000 nationally representative non-institutionalized U.S. civilians. The data is released every two years. The sample does not include persons residing in nursing homes, members of the armed forces or U.S. nationals living abroad (CDC, 2014).

NHANES consists of in-home interviews that include demographic, socio-economic, dietary information [Food Frequency questionnaire FFQ] and health-related questions, followed by health examinations held in a mobile examination center [MEC]. In the MEC, anthropometric measurements, biochemical analysis, dietary data collection [24-hour recall], and physical examinations are obtained (CDC, 2013). The protocol, approved by the NCHS Research Ethics Review Board, requires all participants to give written informed consent. In order to reduce data entry errors and improve interviewer performance, all NHANES data collection, including interviews and examinations, are automated and recorded online with automated edit checks, quality control measurements and questionnaire sequencing (Lee & Nieman, 2009). For the purpose of this study, NHANES data from 2003–2004 and 2005–2006 waves were combined because these were the only two-year cycles that included a measure of serum carotenoids in adults.

3.1.1 Inclusion criteria of subjects

The inclusion criteria for subjects in this study are: all men and women who are 60 years and over and who have complete data on dietary intake, serum carotenoid biomarkers and PF.

Of the total 20,470 participants of NHANES 2003-2006, participants younger than 60 years old [n=16,999], who lacked 24-hour recall [n= 413] or who had all carotenoids serum panels missing [n=153] were excluded. The final analytic cohort included n=2,905 participants. Nevertheless, some participants had zero intake in some of the carotenoid panel, which were excluded to calculate the total dietary intake of carotenoids. There were n=1,829 participants with complete total dietary carotenoids. Also, participants with missing values in a serum carotenoids panel were also excluded to calculate the total serum carotenoid levels resulting in n=2,785 participants with complete data. Participants with available data for total FV intake were n= 1,753. Total intake of carotenoids was calculated using dietary intake of carotenoids plus carotenoids intake from supplements among n=1829.

3.2 Primary outcome

3.2.1 Physical function questionnaire

In NHANES, participants were interviewed about PF ability using the physical function questionnaire [PFQ], which is an instrument designed to ascertain the level of dependence in performing daily tasks. PFQ was administered at home before the physical examination in MEC, using Computer-Assisted Personal Interviewing [CAPI]. Moreover, PFQ collects self-reported data on functional limitations caused by long-term physical, mental and emotional problems or illness (NHANES, 2013a). The PFQ consisted of questions on the level of difficulty of doing several basic and complex daily activities when the participants were by themselves and did not have the use of aids.

In this analysis, 16 questions were included and categorized into three domains, as suggested by NCHS for analytical purposes: ADLs, IADLs, and MD (Altman B, 2008). The first domain of the questionnaire included queries related to four ADLs: 1) transferring or walking from

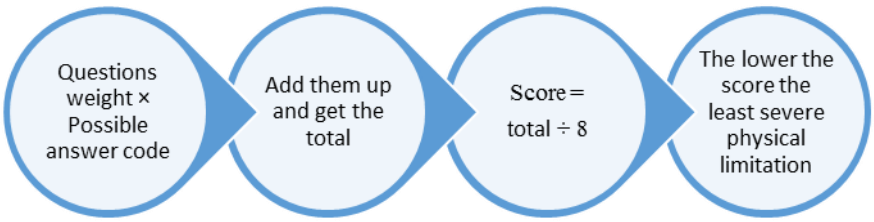
one room to another on the same level; 2) getting in or out of bed; 3) eating, holding a fork, cutting food or drinking from a glass; and 4) dressing, including tying shoes, working zippers and doing buttons. The second domain of the questionnaire included queries related to four IADLs: 1) managing money; 2) doing chores around the house such as vacuuming, sweeping, dusting or straightening up; 3) preparing one's own meal; 4) going out to do things like shopping. The third domain of the questionnaire included queries related to eight activities of MD: 1) walking for a quarter of a mile, 2) walking up to 10 steps without resting, 3) stooping, crouching or kneeling, 4) standing or being on one's feet for about two hours, 5) lifting or carrying something as heavy as 10 pounds, 6) sitting for about two hours, 7) reaching over one's head, and 8) using one's fingers to grasp or handle small objects. Possible answers for all of the previous questions included "no difficulty" coded 1, "some difficulty" coded 2, "much difficulty" coded 3, or "unable to do" coded 4. Other possible answers such as "do not do this activity," "refused," or "don't know" were treated as missing data in the analysis as suggested by NCHS.

3.2.2 Categorization and scoring of PFQ

For the domains of ADLs and IADLs, participants were categorized as "no PFL" if they responded negatively to all four questions in each domain and "have PFL" if they responded affirmatively to one or more questions in each domain. Similarly, for the MD domain, participants were considered "having PFL" if any level of difficulty had been reported for one or more of the eight questions; "no PFL" if they reported no difficulty in doing all the eight activities in the MD domain.

In order to calculate the MD score, which is an indication of the severity of the MD, MD questions were coded according to a scoring system recommended by NCHS. Then weighted average scores were created whereby each question was given a weight to reflect how important a

particular function is to maintaining an independent lifestyle. Weight of 1 was given to questions on: 1) sitting for about two hours and 2) stooping, crouching or kneeling. Weight of 2 was given to questions on: 1) standing or being on one's feet for about two hours and 2) lifting or carrying something as heavy as ten pounds. Weight of 3 was given to questions on: 1) walking up to 10 steps without resting and 2) reaching over one's head. Weight of 4 was given to questions on: 1) walking for a quarter of a mile and 2) using one's fingers to grasp or handle small objects. Those with missing data on some of the movement questions were treated as "no difficulty" and received a weight of 1. Finally, individual scores were calculated and each person's response was coded to the level of difficulty and multiplied by the weight of that particular MD question. Those numbers were totaled and then divided by eight. The scores range from 2.5, which reflect no MD at all, to 10 as maximum. Scores greater than 2.5 were divided into quintiles. Scores in the first quintile reflect the least severe problems and range from 2.5 to 2.625. A score greater than 4.25 represents the most severe level of difficulty with movement. Based on all the types and difficulty levels of reported movements, the lower the total score, the less severe the MD.



The coding and scoring procedures produced three categorical dependent variables: ADLs, IADLs, and MD in addition to one continuous variable: MD score. Therefore, three different models of multiple logistic regression and one multiple linear regression model using least square means were performed in order to detect the association between dietary intake and biomarkers of carotenoids and PF.

3.3 Primary predictors

3.3.1 Plasma carotenoids

Blood samples were collected at the MEC. Carotenoids, including α-carotene, β-carotene, lycopene, β-cryptoxanthin, lutein and zeaxanthin, were analyzed using High Performance Liquid Chromatography [HPLC] with photodiode array detection (NHANES, 2013b) and represented as total intake of carotenoids.

3.3.2 Dietary intake of carotenoids

Study participants were administered two 24-hour recall interviews in the 2003–2004 and 2005–2006 waves of NHANES. The primary dietary interview was administered at the MEC and conducted by trained interviewers using USDA's Automated Multiple-Pass Method [AMPM]. A second dietary recall was administered via telephone interview, approximately three to 10 days after the MEC exam (CDC & Control, 2013). However, for this analysis, only the first in-person, 24-hour dietary recall was used. In this study, total dietary intake of carotenoids, consisting of the sum of α-carotene, β-carotene, lycopene, β-cryptoxanthin, lutein and zeaxanthin, was included as an independent variable.

3.3.3 Fruit and vegetable intakes

Serum carotenoids concentrations are considered reliable biomarkers of FV intake. Therefore, it is expected that higher FV intake will be associated with reduced probability of PFL. In this research study, FV intakes were estimated, as well, to detect the association with ADL, IADL, MD and MD scores. In NHANES, all the types of FV were coded using U. S. Department of Agriculture [USDA] food codes, and the amounts were expressed in grams. However, in this analysis the USDA's MyPyramid Equivalents Database [MPED] that corresponds with NHANES 2003-2004 and Food Pattern Equivalents Database [FPED] that corresponds with NHANES 2005-

2006 were used. These datasets included all reported single and multi-ingredient foods and beverages that were separated into components and assigned cup-equivalents according to standard recipes.

In this analysis total fruit intake, including whole fruit and fruit juice, as well as total vegetable intake of all kinds of vegetables, excluding white potatoes and legumes, were merged into the dataset of NHANES 2003-2006 to provide conversion of grams of total FV intakes into cup equivalents. Total FV intake was divided into tertiles, then the association with each domain of PF was determined using logistic regression or linear regression.

3.3.4 Total intake of carotenoids [diet +supplement]

In NHANES 2003-2006, dietary supplements data consist of three datasets which contain information on products: dietary supplements product information [DSPI], dietary supplements ingredient information [DSII], and dietary supplements blend information [DSBI], and an additional two datasets about participant use of supplements. These five files were merged by an identification variable [participant sequence number or supplement identification number]. Carotenoids supplementation was extracted and calculated as mcg/day consumption. Total carotenoids intake was calculated by adding the dietary intake and supplements intake.

3.4 Covariates

In order to determine the association between dietary intake and biomarkers of carotenoids with PFL, potential confounders, such as sociodemographic and economic characteristics, medical history, lifestyle factors, anthropometric measurements other nutrients, and biomarkers, were examined.

3.4.1 Sociodemographic and economic characteristics

During the household interview, sociodemographic and economic information was obtained. Variables included age, gender, race/ethnicity and education. Race/ethnicity includes [Non-Hispanic white [NHW] [n=1769], Non-Hispanic Black [NHB] [n=486], and Mexican American [MA], which consists of MA, other Hispanic and other ethnicities [n=650]]. For education level, participants were categorized as less than high school [n= 1089], completed high school [n= 748], and some college and above [n=1068].

3.4.2 Anthropometric measurements

The NHANES anthropometry is conducted in MEC. Height and weight were measured and body mass index [BMI] was calculated for all adults.

3.4.2.1 Weight

To accurately weigh participants, only underpants were worn beneath the standard MEC examination gown, which consisted of a disposable shirt, pants and slippers. Participants were weighed in kilograms using a digital weight scale. Participants stood in the center of the scale platform, hands at sides and looking straight ahead (CDC & NHANES, 2013).

3.4.2.2 Height.

Height was measured using a stadiometer with a fixed vertical backboard and an adjustable head piece. Each participant was asked to untie their hair and remove anything from the top of their head, stand up straight with body weight evenly distributed, and place both feet flat on the platform with heels together and toes apart, and place back of the head, shoulder blades, buttocks and heels in contact with the backboard (CDC & NHANES, 2013).

3.4.2.3 Body Mass Index

Body mass index [BMI] is calculated as weight in kilograms divided by height in meter-squared. BMI categories include: underweight [BMI values < 18.5], healthy weight [BMI values 18.5-24.9], overweight [BMI values 25.0-29.9], and obese [BMI values > 30.0] (CDC & NHANES, 2013).

3.4.2.4 Lean body mass

Dual-energy X-ray absorptiometry [DXA] scans are used to evaluate total body fat, non-bone lean tissue and bone lean tissue, which can be obtained with a high degree of accuracy (Rothney, Brychta, Schaefer, Chen, & Skarulis, 2009). In NHANES, lean body mass, excluding bone mineral content, was derived from DXA and added to the models.

3.4.3 Medical history and lifestyle factors

During the in-home interview, answers to questions on self-reported medical histories were obtained as well as lifestyle factors such as smoking, alcohol consumption and PA as discussed below.

3.4.3.1 Medical history

Chronic conditions and comorbidities such as cardiovascular disease, cancer, diabetes, arthritis and osteoporosis are strongly associated with development of PFL (Bhattacharya, Choudhry, & Lakdawalla, 2008; Dunlop, Manheim, Sohn, Liu, & Chang, 2002; Freedman, Schoeni, Martin, & Cornman, 2007) and considered potential confounders in the decline of PF. Self-reported diagnosis of these chronic diseases was controlled for in the regression models. These health problems include arthritis/rheumatism, osteoporosis, diabetes and cardiovascular diseases [e.g. hypertension, stroke, heart attack, angina, coronary heart disease, congestive heart failure, and high cholesterol] (NHANES, 2013a). In addition, self-reported health status [excellent, very good, and good versus fair and poor] has been added to the models.

3.4.3.2 Cigarette smoking and alcohol intake

Smoking status affects antioxidant vitamin levels in the serum (Kuo, Al Snih, Kuo, & Raji, 2012; Valachovicova, Krajcovicova-Kudlackova, Ginter, & Paukova, 2003; Voutilainen et al., 2006). In NHANES, respondents were asked whether they had smoked at least 100 cigarettes in their entire lives. Smoking status is categorized into "non-smokers" if they reported never having smoked 100 cigarettes during their lifetime. Those who answered "yes" were asked whether they now smoke cigarettes every day, some days or not at all. For our analysis, "current smokers" are those who had smoked at least 100 cigarettes during their lifetime and reported smoking either every day or some days. "Former smokers" are those who reported smoking at least 100 cigarettes during their lifetime, but currently did not smoke (Alshaarawy, Xiao, Andrew, Burchfiel, & Shankar, 2013; Kahende, Adhikari, Maurice, Rock, & Malarcher, 2009).

Another confounder is the consumption of alcohol. Excessive use of alcoholic beverages is inversely associated with serum carotenoids (Stuck et al., 1999; Woodside, Young, Gilchrist, et al., 2013),The *Dietary Guidelines for Americans* defines moderate drinking as no more than one drink per day for women and no more than two drinks per day for men (Services, 2010). In NHANES, participants were asked whether they had any alcoholic drinks in the past 12 months. Those who responded "no" were classified as "non-drinkers." For those who responded "yes," further questions were administered about the average number of alcoholic drinks per day. In this analysis, men who have 1 to 2 drinks per day and women who have 1 drink per day are classified as "moderate drinkers." "Heavy drinkers" are men who have 3 or more drinks and women who have 2 or more drinks per day.

3.4.3.3 Physical activity

Epidemiological studies have demonstrated an inverse association between PA and PFL (Hillsdon, Brunner, Guralnik, & Marmot, 2005; Manini & Pahor, 2009). In NHANES, self-reported PA was assessed

during the household interview. It consisted of a series of questions that required participants to recall PA behaviors during the past 30 days. Questions focused on the frequency and duration of PA performed during household, transportation and leisure activities.

Estimated intensity levels were assigned, as metabolic equivalent of task [MET], to all reported household activities [4.5 METs] and transportation activities [4.0 METs] as suggested by Compendium of Physical Activities (Tucker, Welk, & Beyler, 2011). For leisure-time PA, participants were asked to report the level of activity exertion [moderate or vigorous] for each reported activity. Each leisure activity was assigned a MET score based on the level of exertion. For example, moderate-intensity cycling was considered 4 METs and vigorous-intensity cycling was considered 8 METs.

In this analysis, PA levels were calculated using MET-minutes per week. Total MET-minutes of PA are calculated by multiplying the weekly PA volume [duration and frequency] of each activity by its corresponding MET value. Participants are categorized into one of the following three guideline-based activity levels according to their total weekly MET-minutes: "none" if participant has 0 MET-minutes per week; "insufficient" to meet the guidelines if participant has 1 to 499 MET-minutes per week; and "sufficient" to meet the guidelines if participant has 500 and more MET-minutes per week (Sahni et al., 2009).

3.4.4. Other serum biomarkers.

3.4.4.1 Nutritional biomarkers

Several vitamins have antioxidant properties that may act synergistically with carotenoids in reducing the risk of PFL such as vitamin C and vitamin E. These were examined as confounders in the regression models. In NHANES, vitamin C was measured using isocratic HPLC with electrochemical detection at 650 mV1. In addition, serum concentrations of vitamin E were measured using HPLC with photodiode array detection (NHANES, 2013b).

Another nutritional biomarker considered as a potential confounder is serum vitamin D [25-OH-D]. Recent studies have shown that lower serum 25-OH-D status is associated with poorer functional mobility decreasing muscle mass and muscle strength (Gschwind, Bischoff-Ferrari, Bridenbaugh, Hardi, & Kressig, 2014). In NHANES, 25-OH-D was analyzed using RIA Dias Orin [formerly Incstar] 25-OH-D assay (Baldrick et al., 2011; NHANES, 2013b).

Furthermore, lipid profile is important to consider in this analysis due to its association with carotenoids level and physical function. Lipid profile such as triglycerides [TG], LDL- and High DL-cholesterol is included in this analysis. In NHANES, TG concentration was determined in the serum using the Dade Behring BN100 Nephelometer method or the Beckman Synchron latex-enhanced nephelometry 20. The normal level of TG should be less than 150 mg/dl [less than 1.7 mmol/l] (Baldrick et al., 2011; NHANES, 2013b). The HDL-cholesterol concentration was determined enzymatically by cholesterol esterase and cholesterol oxidase. HDL-cholesterol and total cholesterol values are used to calculate the LDL-cholesterol level based on Fried Ewald formula [LDL cholesterol [mmol/L] = Total cholesterol – HDL cholesterol – Total triglyceride ÷ 2.19] (Baldrick et al., 2011; NHANES, 2013b).

3.4.4.2. Inflammatory biomarkers

A chronic inflammatory state may be detrimental by accelerating the progression of medical conditions that result in functional decline and disability. In fact, an accelerated decline in muscle mass and strength with aging is probably one of the major causes of disability among older adults (Cesari, Penninx, et al., 2004). In NHANES, CRP is measured using the Nephelometry method, which quantifies CRP by latex-enhanced nephelometry and is controlled for in this analysis.

Uric acid is a constituent of the cell cytosol generated by nucleotide catabolism, which reflects cell death and turnover. In NHANES, uric acid level was measured by oxidization with

uricase to form allantoin and H2O2 using Hitachi Model 737 Multichannel Analyzer, Boehringer Mannheim Diagnostics (NHANES, 2013b).

3.4.5. Other dietary intake estimates

Energy intake, protein and fat consumption are potential confounders. These dietary intakes have putative associations with carotenoid levels in the serum, which may indirectly influence the association between carotenoids and physical function (Barbara Marzani, 2004; Fano et al., 2001). Several studies suggested that increasing protein intake is a simple strategy to slow the age-related loss of muscle proteins (D. Paddon-Jones & Leidy, 2014; Douglas Paddon-Jones et al., 2008) (Mosoni et al., 2014). Therefore, dietary protein intake is included in the analysis as a confounder that may affect PFL.

Dietary fat intake is hypothesized to be an important factor for carotenoids' bioavailability and absorption since carotenoids are fat-soluble antioxidants (Unlu, Bohn, Clinton, & Schwartz, 2005). Therefore, dietary fat is examined as a potential confounder.

Moreover, to adjust for overall food intake, the analysis must be adjusted for daily dietary energy intake using a nutrient residual method (Willett, Howe, & Kushi, 1997). Absolute and relative nutrient intake values are reported and categorized into quintiles to provide comparisons for the likelihood of greater functional limitations.

3.5 Statistical Analysis

Data analyses were performed using SUDAAN callable SAS version 11.0, which adjusts for complex survey design. Datasets from NHANES 2003–2004 and 2005–2006 waves were combined according to NHANES analytic guidelines. Statistical significance was assessed with two-sided *P* values at α level of 0.01 for the entire analysis. In this analysis, selected variables from demographic, dietary, examination, laboratory and questionnaire files were included. Variables were assessed for normality by examination of histograms and Q-Q plot, and log

transformation was done for MD score, triglyceride, dietary intake of total carotenoids and adjusted dietary intake of total carotenoids, total intake of carotenoids, and CRP as needed to perform t-tests while comparing individuals with PFL and with no PFL. In addition, outliers, or observation points that are distant from other observations, were checked.

Descriptive analyses were obtained for each variable of interest and reported by physical limitation status [Table 2] and by gender and PF domains [Table 3]. Continuous variables were summarized using means and standard error. However, median was reported for skewed variables such as MD score, triglyceride, dietary intake of total carotenoids and adjusted dietary intake of total carotenoids, total intake of carotenoids and CRP. Categorical variables such as gender, race/ethnicity, smoking status and PA level were presented as percentages. Continuous and categorical variables were analyzed using t-test and chi-square, respectively. For the skewed variables, t-test was performed on transformed form.

Multiple logistic regression models were used to assess the association between dietary intake of carotenoids, serum concentration of carotenoids and total FV intake with PFL within each domain using different models to adjust for potential confounders. Simple and adjusted linear regression using least square means were performed to identify the mean difference of MD score outcome according to quintiles of dietary intake and plasma levels of carotenoids and tertiles of FV intake and total intake.

In order to perform multiple logistic regression and multiple linear regression, results of collinearity tests and stepwise methods were used to determine inclusion of previously discussed covariates in the models. Also, pseudo R-squared and the significance of the overall models using Wald-F test were considered to determine how well the models fit the data. However, all models were adjusted for sociodemographic variables [age, gender, race/ethnicity, and education] in the

second and full adjusted model regardless of their significance status. However, other previously mentioned covariates confounders were included or excluded based on the stepwise method.

3.5.1 Analysis

Research question 1: What are the characteristics of the study population with and without PFL?

For continuous variables with normal distribution, mean and SE were reported. However, for skewed continuous variables, median and SE were reported. Skewed variables such as absolute and relative dietary intake and total intake of carotenoids, MD score, TG, and CRP were log transformed. To compare participants with and without PFL, a t-test was used on the normally distributed data and on the transformed variables. For categorical variables, the number of participants and percentages were reported. Chi-square analysis were used to compare participants with and without PFL.

Research question 2: Is there an association between serum level of carotenoids and physical functional limitations of ADL, IADL, MD and MD scores among older adults?

- *Model 1*
  - *Main predictor variables:* quintile of serum carotenoids
  - *Outcome variables:* ADL, IADL, MD and MD scores with each outcome in a separate model
- *Model 2*
  - Confounding variables:
    - *Categorical variables:* Race/ethnicity, gender and education
    - *Continuous variables:* Age
- *Model 3 for ADL and IADL*
  - Confounding variables for the third model: variables from model two plus the following:
    - *Categorical variables:* BMI, PA level, self-reported health status, having arthritis
- *Model 3 for MD and MD score*
  - Confounding variables for the third model: variables from model two plus the following:
    - *Categorical variables:* BMI, PA level, self-reported health status, having arthritis
    - *Continuous variables:* C- reactive protein

Research question 3: Is there an association between energy-adjusted and unadjusted dietary intake of carotenoids and physical functional limitations of ADL, IADL, MD and MD scores among older adults?

- *Model 1*

- *Main predictor variables:* quintiles of dietary intake of total carotenoids
- *Outcome variables:* ADL, IADL, MD and MD scores, each outcome in a separate model
- *Model 2*
- Confounding variables for the second model:
    - *Categorical variables:* Race/ethnicity, gender and education
    - *Continuous variables:* Age
- *Model 3*
- Confounding variables for the third model: variables from model two plus the following:
    - *Categorical variables:* BMI, PA level, and self-reported health status

Research question 4: Is there an association between total intake [diet + supplement] of carotenoids and physical functional limitations of ADL, IADL, MD and MD scores among older adults?

- *Model 1*
- *Main predictor variables:* tertile of total intake of carotenoids
- *Outcome variables:* ADL, IADL, MD and MD score, each outcome in a separate model.
- *Model 2*
- Confounding variables for the second model:
    - *Categorical variables:* Race/ethnicity, gender and education
    - *Continuous variables:* Age
- *Model 3*
- Confounding variables for the third model: variables from model two plus the following:
    - *Categorical variables:* BMI, PA level, and self-reported health status
    - *Continuous variables:* Total energy intake.

Research question 5: Is there an association between total FV intake and physical functional limitations of ADL, IADL, MD and MD scores among older adults?

- *Model 1*
- *Main predictor variables:* tertile of fruit and vegetable intake
- *Outcome variables:* ADL, IADL, MD and MD score, each outcome in a separate model
- *Model 2*
- Confounding variables for the second model:
    - *Categorical variables:* Race/ethnicity, gender and education
    - *Continuous variables:* Age
- *Model 3*
- Confounding variables for the third model: variables from model two plus the following:
    - *Categorical variables:* BMI, PA level, self-reported health status, having diabetes
    - *Continuous variables:* Total energy intake and vitamin C

Chapter 4: Results

4.1 Characteristics of the study population

This study included a total of 2,905 participants, 1,469 [50.6%] men and 1,436 [49.4%] women. The mean [± SE] age of the study population was 70.9 ±0.2 years [70.4 ±0.2 years for men and 71.3 ± 0.3 years for women]. Non-Hispanic white men and women constituted the largest proportion of the study population [61.6% and 60.2%, respectively]. More than one-third of the study population has some college education. Of the study sample, 1,960 [67.5%] participants had one or more limitation in PF domains. A larger proportion of women reported difficulties in ADL, IADL and MD compared to men [table1].

Table 1

Characteristics of the study population by gender

Characteristics	(n)	Men	Women
Gender n (%)	2905	1469 (50.6)	1436 (49.4)
Age (mean ±SE)	2905	70.4 ±0.2	71.3 ±0.3
Race/Ethnicity n (%)	2905		
Non-Hispanic White		905 (61.6)	864 (60.2)
Non-Hispanic Black		247 (16.8)	239 (16.6)
Mexican American		317 (21.6)	333 (23.2)
Education n (%)	2905		
Less than high school		552 (37.6)	537 (37.4)
Completed high school		336 (22.9)	412 (28.7)
Some college and above		581 (39.6)	487 (33.9)
Have at least one physical functional limitation (yes) n (%) [a]	2905	904 (61.5)	1056 (73.5)
Have ADLs limitation (yes) n (%) [b]		359 (24.4)	383 (26.7)
Have IADLs limitation (yes) n (%) [c]		386 (26.5)	577 (40.3)
Have MD (yes) n (%) [d]		876 (59.6)	1040 (72.4)
MD score among people with PFL (mean ±SE) [e]		3.56 ±0.04	3.85 ±0.05

[a] Participants who self-reported difficulty in one or more of the PF domains, which are: activities of daily living (ADL), instrumental activities of daily living (IADL) and movement difficulty (MD).
[b] Participants who reported difficulties in one or more of the ADLs questions
[c] Participants who reported difficulties in one or more of the IADLs questions
[d] Participants who reported difficulties in one or more of the MD questions
[e] MD score: is a score to measure the severity of Movement difficulty, ranges from 2.5 to 10. The higher the score the, the higher the severity.
Abbreviation*:* (SE) = standard error; PFL = physical functional limitation

Participants with no PFL in any of the PF domains were relatively younger, had higher educational attainment and were more likely to self-rate their health as excellent to good. Serum concentrations of total carotenoids were significantly lower among individuals with PFL. Participants with PFL had significantly higher BMI but significantly lower lean body mass than those with no PFL and were significantly more inactive. They also had significantly more chronic conditions: Of study participants with self-reported histories of chronic diseases, cardiovascular disease and arthritis appear to be the most common chronic condition among those with PFL. Compared to participants with no PFL, those with at least one limitation had significantly fewer FV and consumed significantly less carotenoids. When adjusted by calorie intake, the difference in carotenoids intake was no longer significant [table 2]. Of the 2,905 study participants with self-reported histories of difficulties in PF, ADL limitations were observed for 359 [24.4%] men and 383 [26.7%] women. IADL limitations were observed for 386 [26.5%] men and 577 [40.3%] women, and MD were observed for 876 [59.6%] men and 1,040 [72.4%] women [table 3]. More details about the characteristics of the study population are summarized in the appendix [tables 3 - 5].

Table 2
Characteristics by physical functional limitation (PFL) [a]

Characteristics	*(n)*	*No PFL* [a]	*(n)*	*PFL* [a] *(1 or more)*	*P* - value [*]
n (%)		945 (32.5)		1960 (67.5)	
Age	945	69.0 ±0.29	1960	71.9 ±0.24	<.0001
MD score [b c]	945	2.50 ±0.01	1960	4.75 ±0.03	<.0001
Dietary carotenoids (mcg / day) [b]	621	8413 ±654.4	1208	7209 ±438.5	0.0388
Adjusted dietary carotenoids (mcg / day) [b d]	621	8309 ±405.6	1208	7623 ±395.7	0.2535
Total carotenoids intake (diet + supplement) (mcg / day) [b]	621	8748 ±623	1208	7375 ±444	0.0512
Total FV intake (cup equivalent / day)	559	2.93 ±0.10	1199	2.63 ±0.06	0.0020
Energy intake (kcal / day)	945	1896 ±36.7	1960	1743 ±21.9	<.0001
Dietary Protein intake (gm / day)	945	73.7 ±1.72	1960	67.4 ±1.04	<.0001
Dietary fat intake (gm / day)	945	72.7 ±1.80	1960	67.8 ±1.13	0.0009
Serum carotenoids (ug/dL)	893	101.6 ±2.67	1892	87.5 ±1.91	<.0001
Serum level of vitamin D (ng/mL)	945	25.3 ±0.43	1960	22.5 ±0.32	<.0001
Serum level of vitamin C (mg / dL)	934	1.14 ±0.02	1948	1.10 ±0.02	0.4348
Serum level of vitamin E (ug/dL)	945	1645 ±25.1	1960	1648 ±31.0	0.9190
LDL (mmol/L)	939	3.13 ±0.04	1952	3.01 ±0.03	0.0109
HDL (mmol/L)	945	1.47 ±0.02	1958	1.43 ±0.01	0.9167
TG (mmol/L) [b]	939	1.35 ±0.04	1954	1.47 ±0.03	0.1111
Uric acid (mg/dL)	940	5.53 ±0.06	1954	5.71 ±0.04	0.0050
C-reactive protein (mg/dL) [b]	945	0.18 ±0.01	1959	0.26 ±0.01	0.0001
BMI (kg/m^2)	938	27.2 ±0.15	1893	29.1 ±0.16	<.0001
Lean body mass (kg)	903	47.1 ±0.48	1864	45.9 ±0.42	0.0224
Told by doctor to have (yes) n (%)	945		1960		
CVD		660 (29.1)		1607 (70.9)	<.0001
Cancer		152 (16.1)		382 (19.50)	0.0264
Diabetes		135 (14.3)		515 (26.3)	<.0001
Arthritis		271 (28.7)		1191 (60.8)	<.0001
Osteoporosis		74 (7.83)		336 (17.2)	<.0001
Physical activity n (%) [e]	945		1960		
Sufficiently active		489 (51.8)		642 (32.8)	<.0001
Insufficiently active		92 (9.74)		205 (10.5)	<.0001
Inactive		364 (38.5)		1113 (56.8)	<.0001

Note: Values are means ±SE or percentage if stated.
[*] P-values are for Student's t-test for continuous variables and for Chi-square test for categorical variables comparing between participants with and without PFL. Significance level is $p < 0.01$
[a] PFL: physical functional limitations -- if participants reported difficulty in one or more of the PF domains.
No PFL: if participants reported no difficulty in all of the PF domains, which are: activities of daily living, instrumental activities of daily living and movement difficulty.
[b] Values are expressed as median ±SE due to right skewness and P values of Student's t-test for log-transformed variables.
[c] MD score: is a score measuring the severity of upper and lower movement difficulty, ranges from 2.5 to 10. The higher the score, the higher the severity.
[d] Adjusted for total energy intake using the residual method suggested by Walter Willett (Willett, 1997).
[e] Physical activity: calculated using metabolic equivalent of task (MET) minutes per week as suggested by physical activity guidelines for Americans. Defined as inactive if participant has 0 MET-minutes per week; "insufficient" to meet the guidelines if participant has 1 to 499 MET-minutes per week; and "sufficient" to meet the guidelines if participant has 500 and more MET-minutes per week.
Abbreviations - MD: Movement difficulty; FV: Fruit and vegetable; LDL: Low density lipoprotein; HDL: High density lipoprotein; TG: triglyceride; BMI: Body mass index; and CVD: cardiovascular disease.

4.2 Association between serum concentration of total carotenoids and PF domains

There were 2,785 participants who had complete serum carotenoids data. The association between serum carotenoids and PF domains are shown in table 6. Results from the regression analysis show significant inverse associations between serum carotenoids and risk of limitation in ADL, IADL and MD. Once the models were adjusted for confounders [model 3], the associations were attenuated but remained significant for all domains particularly in the three lowest quintiles compared to the highest quintile. Using pseudo R-squared, the variables in the model explained 24%, 32%, and 29% of the data, respectively. The associations were significant [p for trend < 0.01] for all models when carotenoids were examined as a continuous variable, although highly attenuated particularly in the relationship between ADL and serum carotenoids and MD and serum carotenoids [p for trend = 0.02 and p for trend = 0.01, respectively].

Table 6

Associations between total serum carotenoids and PF domains using logistic regression analysis modeling

Model	R^2	PF domains	Quintiles for serum concentration of total carotenoids OR (95%CI)					
			1st Quintile (≤ 51.9 ug/dL) n=559	2nd Quintile (52 - ≤ 70.6 ug/dL) n=555	3rd Quintile (70.7 - ≤ 90.8 ug/dL) n=557	4th Quintile (90.9 - ≤ 118.6 ug/dL) n=557	5th Quintile [e] (> 118.6 ug/dL) n=557	*P* for trend [*]
		ADL limitation						
1[a]			3.20 (2.27 - 4.50)	2.12 (1.42 - 3.17)	2.11 (1.57 - 2.84)	1.35 (0.97 - 1.88)	1.00	0.0000
2[b]			3.20 (2.15 - 4.77)	2.06 (1.31 - 3.23)	2.07 (1.47 - 2.92)	1.34 (0.93 - 1.95)	1.00	0.0001
3[c]	0.24		2.07 (1.19 - 3.61)	1.47 (0.88 - 2.44)	1.89 (1.29 - 2.77)	1.28 (0.84 - 1.95)	1.00	0.0198
		IADL limitation						
1[a]			2.99 (2.19 - 4.08)	2.15 (1.55 - 2.99)	1.66 (1.22 - 2.27)	1.57 (1.20 - 2.06)	1.00	0.0000
2[b]			3.40 (2.50 - 4.63)	2.25 (1.55 - 3.26)	1.75 (1.26 - 2.44)	1.66 (1.22 - 2.25)	1.00	0.0000
3[c]	0.32		2.29 (1.58 - 3.32)	1.69 (1.17 - 2.44)	1.44 (0.97 - 2.14)	1.68 (1.13 - 2.50)	1.00	0.0009
		MD						
1[a]			2.84 (2.04 - 3.96)	2.31 (1.66 - 3.20)	1.29 (0.96 - 1.74)	1.48 (1.06 - 2.05)	1.00	0.0000
2[b]			3.47 (2.51 - 4.80)	2.64 (1.95 - 3.57)	1.44 (1.08 - 1.92)	1.62 (1.16 - 2.26)	1.00	0.0000
3[d]	0.29		1.95 (1.32 - 2.89)	1.75 (1.23 - 2.50)	1.02 (0.70 - 1.49)	1.41 (0.97 - 2.06)	1.00	0.0112

Adjusted covariates:
[a] Model 1= No adjustment
[b] Model 2 = Age, gender, race and education
[c] Model 3 = Model 2 + Body mass index, physical activity, self-rated health status, and having arthritis.
[d] Model 3 = Model 2 + Body mass index, physical activity, self-rated health status, having arthritis and C-reactive protein.
[e] Quintiles cut-off point of carotenoids concentrations and the number of subjects in each quintile.

Abbreviations – PF: physical function; ADL: activities of daily living; IADL: instrumental activities of daily living; MD: movement difficulty; OR: odds ratio; CI: confidence interval.
ORs are odds ratios of limitation in physical function domains (ADL, IADL, and MD) comparing participants in the lower quintiles of serum concentration of total carotenoids to the highest quintile.
[*] P-value for trend of the odds of having difficulties in ADL, IADL, and MD across serum concentrations of total carotenoids quintiles <0.01.

4.3 Associations between unadjusted and adjusted dietary intake, total intake of carotenoids and PF domains

There were 1,829 participants with complete dietary intake data for carotenoids. The associations between dietary intakes of carotenoids and PF domains are shown in table 7. The correlation between serum and dietary carotenoids was somewhat weak although the p-value was highly significant (r=0.34 and p=0.0001). There was an inverse association between carotenoids intake and risk of IADL limitation [p for trend 0.001] even after controlling for confounders [model

3]. Based on the pseudo R-squared, this model explained 28% of the data. These associations remained the same although attenuated when carotenoids were adjusted for energy intake [table 8]. So, absolute and relative intake were associated with limitation in at least one IADL. The associations were also similar when carotenoids from diet and supplement were examined in association with PFL [table 9]. No associations were observed between carotenoids and the other PF domains.

Table 7

Association between dietary intake of carotenoids and PF domains using logistic regression analysis modeling

Model	R^2	PF domains	Quintiles for dietary intake of carotenoids OR (95%CI)					
			1st Quintile (≤ 2779 mcg) n=366	2nd Quintile (2779.1 - ≤ 5301 mcg) n=366	3rd Quintile (5301.1 - ≤ 9885 mcg) n=366	4th Quintile (9885.1 - ≤ 17809 mcg) n=366	5th Quintile [d] (> 17809 mcg) n= 365	*P* for trend [*]
		ADL limitation						
1[a]			1.26 (0.84 - 1.88)	1.31 (0.87 - 1.98)	1.21 (0.78 - 1.88)	0.98 (0.60 - 1.60)	1.00	0.3379
2[b]			1.11 (0.75 - 1.63)	1.17 (0.77 - 1.78)	1.14 (0.74 - 1.77)	0.92 (0.56 - 1.51)	1.00	0.6497
3[c]	0.18		0.84 (0.57 - 1.24)	0.98 (0.59 - 1.64)	1.04 (0.67 - 1.61)	0.81 (0.49 - 1.35)	1.00	0.6247
		IADL limitation						
1[a]			2.72 (1.99 - 3.73)	2.15 (1.48 - 3.13)	1.91 (1.26 - 2.90)	2.15 (1.24 - 3.71)	1.00	0.0000
2[b]			2.33 (1.72 - 3.17)	1.91 (1.29 - 2.81)	1.72 (1.12 - 2.67)	2.03 (1.13 - 3.66)	1.00	0.0003
3[c]	0.28		2.00 (1.40 - 2.85)	1.79 (1.16 - 2.78)	1.68 (1.06 - 2.66)	2.03 (1.11 - 3.70)	1.00	0.0011
		MD						
1[a]			1.32 (0.95 - 1.83)	1.38 (0.97 - 1.96)	1.03 (0.68 - 1.58)	1.16 (0.85 - 1.60)	1.00	0.1653
2[b]			1.13 (0.80 - 1.61)	1.20 (0.84 - 1.70)	0.88 (0.57 - 1.36)	1.05 (0.75 - 1.48)	1.00	0.6978
3[c]	0.26		0.90 (0.65 - 1.25)	1.06 (0.69 - 1.62)	0.84 (0.55 - 1.28)	0.98 (0.69 - 1.39)	1.00	0.6895

Adjusted covariates:
[a] Model 1= No adjustment.
[b] Model 2 = Age, gender, race and education
[c] Model 3 = Model 2 + Body mass index, Physical activity, and self-rated health status.
[d] Quintiles cut-off point of dietary intake of carotenoids and the number of subjects in each quintile.

Abbreviations – ADL: activities of daily living; IADL: instrumental activities of daily living; MD: movement difficulty; OR: odds ratio; CI: confidence interval.
ORs are odds ratios of limitation in physical function domains (ADL, IADL, and MD) comparing participants in the lower quintiles of dietary intake of total carotenoids to the highest quintile.
[*] P-value for trend of the odds of having difficulties in ADL, IADL, and MD across dietary intake of total carotenoids quintiles <0.01.

Table 8

Association between adjusted dietary intake of carotenoids and PF domains using logistic regression analysis modeling [a]

Model	R^2	PF domains	Quintiles for adjusted dietary intake of carotenoids OR (95%CI)					
			1st Quintile (≤ 3415 mcg) n=365	2nd Quintile (3415.1- ≤ 6064 mcg) n=367	3rd Quintile (6064.1 - ≤ 9910 mcg) n=365	4th Quintile (9910.1 - ≤ 17264 mcg) n=366	5th Quintile [d] (> 17264 mcg) n=366	*P* for trend [*]
		ADL limitation						
1[a]			0.87 (0.57 - 1.34)	1.16 (0.78 - 1.73)	1.33 (0.79 - 2.25)	1.05 (0.62 - 1.79)	1.00	0.6378
2[b]			0.83 (0.56 - 1.24)	1.07 (0.70 - 1.63)	1.24 (0.75 - 2.06)	1.01 (0.59 - 1.73)	1.00	0.8790
3[c]	0.19		0.68 (0.45 - 1.04)	0.94 (0.61 - 1.44)	1.26 (0.75 - 2.13)	0.88 (0.50 - 1.54)	1.00	0.6493
		IADL limitation						
1[a]			1.69 (1.30 - 2.20)	1.91 (1.28 - 2.86)	1.77 (1.21 - 2.59)	1.96 (1.24 - 3.11)	1.00	0.0001
2[b]			1.79 (1.38 - 2.32)	1.64 (1.05 - 2.56)	1.58 (1.12 - 2.24)	1.80 (1.07 - 3.02)	1.00	0.0009
3[c]	0.28		1.58 (1.14 - 2.19)	1.51 (0.92 - 2.49)	1.66 (1.21 - 2.28)	1.73 (1.07 - 2.81)	1.00	0.0021
		MD						
1[a]			1.26 (0.91 - 1.75)	1.32 (0.91 - 1.91)	1.32 (0.91 - 1.91)	1.26 (0.88 - 1.80)	1.00	0.0600
2[b]			1.32 (0.94 - 1.85)	1.15 (0.79 - 1.69)	1.15 (0.80 - 1.66)	1.14 (0.76 - 1.72)	1.00	0.2277
3[c]	0.26		1.19 (0.86 - 1.66)	0.99 (0.66 - 1.49)	1.15 (0.78 - 1.67)	1.05 (0.71 - 1.57)	1.00	0.5418

[a] Adjusted dietary intake of total carotenoids: adjusted for total energy intake using the residual method suggested by Walter Willett (Willett, 1997, Adjustment for total energy intake in epidemiologic studies).
Adjusted covariates:
[a] Model 1= No adjustment
[b] Model 2 = Age, gender, race and education
[c] Model 3 = Model 2 + Body mass index, physical activity, and self-rated health status.
[d] Quintiles cut-off point of adjusted dietary intake of carotenoids and the number of subjects in each quintile.

Abbreviations – ADL: activities of daily living; IADL: instrumental activities of daily living; MD: movement difficulty; OR: odds ratio; CI: confidence interval. ORs are odds ratios of limitation in physical function domains (ADL, IADL, and MD) comparing participants in the lower quintiles of adjusted dietary intake of total carotenoids to the highest quintile.
[*] P-value for trend of the odds of having difficulties in ADL, IADL, and MD across adjusted dietary intake of total carotenoids quintiles <0.01

Table 9
Association between total intake of carotenoids from diet and supplement and PF domains using logistic regression analysis modeling

Model		PF domains	Quintiles for total intake of carotenoids from diet and supplement OR (95%CI)					
			1st Quintile (≤ 2911 mcg) n=366	2nd Quintile (2911.1 - ≤ 5579 mcg) n=366	3rd Quintile (5579.1 - ≤ 10080 mcg) n=366	4th Quintile (10080.1 - ≤ 17914 mcg) n=366	5th Quintile [d] (> 17914 mcg) n=365	*P* for trend [*]
		ADL limitation						
1[a]			1.32 (0.89 - 1.96)	1.15 (0.76 - 1.76)	1.27 (0.80 - 2.02)	1.05 (0.64 - 1.74)	1.00	0.3096
2[b]			1.15 (0.79 - 1.67)	1.04 (0.69 - 1.57)	1.18 (0.75 - 1.86)	0.99 (0.60 - 1.65)	1.00	0.6267
3[c]	0.21		0.82 (0.52 - 1.29)	0.84 (0.50 - 1.41)	1.16 (0.71 - 1.88)	0.82 (0.50 - 1.37)	1.00	0.5910
		IADL limitation						
1[a]			2.65 (1.93 - 3.64)	2.06 (1.41 - 3.01)	1.75 (1.11 - 2.73)	1.98 (1.17 - 3.36)	1.00	0.0001
2[b]			2.24 (1.64 - 3.06)	1.85 (1.25 - 2.73)	1.56 (0.99 - 2.46)	1.85 (1.04 - 3.29)	1.00	0.0009
3[c]	0.29		1.76 (1.16 - 2.67)	1.68 (1.09 - 2.60)	1.50 (0.93 - 2.43)	1.75 (1.00 - 3.06)	1.00	0.0069
		MD						
1[a]			1.31 (0.95 - 1.80)	1.34 (0.94 - 1.90)	1.15 (0.77 - 1.72)	1.10 (0.79 - 1.52)	1.00	0.1468
2[b]			1.12 (0.79 - 1.58)	1.18 (0.83 - 1.69)	0.97 (0.65 - 1.44)	0.99 (0.69 - 1.41)	1.00	0.6708
3[c]	0.26		0.92 (0.67 - 1.27)	1.11 (0.72 - 1.70)	0.95 (0.64 - 1.42)	0.94 (0.66 - 1.35)	1.00	0.8756

Note: Total intake of carotenoids includes carotenoids intake from diet + supplement
Adjusted covariates:
[a] Model 1= No adjustment
[b] Model 2 = Age, gender, race and education
[c] Model 3 = model 2 + Body mass index, physical activity, self-rated health status, and total energy intake.
[d] Quintiles cut-off point of total intake and the number of subjects in each quintile.

Abbreviations – ADL: activities of daily living; IADL: instrumental activities of daily living; MD: movement difficulty; OR: odds ratio; and CI: confidence interval.
ORs are odds ratios of limitation in physical function domains (ADL, IADL, and MD) comparing participants across the quintile of total intake of carotenoids.
[*] P-value for trend of the odds of having difficulties in ADL, IADL, and MD across total intake of total carotenoids quintiles <0.01

4.4 Associations between fruit and vegetable intakes and PF domains

There were 1,758 participants with complete data on FV consumption. The associations between FV intake and PF domains are shown in table 10. In the unadjusted model, there was an association between FV intake in the lowest two tertiles compared to the highest intake and limitation in IADL only. However, after adjustment for confounders, the odds of having limitation in IADL were no longer significant. There were no significant associations between increasing FV intake and other PF domains.

Table 10
Association between fruit and vegetable intake and PF domains using logistic regression analysis modeling

Model	R^2	PF domains	Tertile for fruit and vegetable intake			
			OR (95%CI)			
			1st Tertile (≤ 1.65 cup) n=581	2nd Tertile (1.66 - ≤ 3.043 cup) n=581	3rd Tertile [d] (> 3.043 cup) n=596	*P* for trend [*]
		ADL				
1[a]			1.48 (0.97 - 2.25)	1.17 (0.82 - 1.67)	1.00	0.1346
2[b]			1.35 (0.89 - 2.04)	1.10 (0.76 - 1.59)	1.00	0.2868
3[c]	0.23		0.98 (0.63 - 1.53)	0.89 (0.59 - 1.35)	1.00	0.7224
		IADL				
1[a]			1.96 (1.34 - 2.86)	1.39 (1.04 - 1.84)	1.00	0.0017
2[b]			1.71 (1.13 - 2.58)	1.28 (0.95 - 1.72)	1.00	0.0174
3[c]	0.31		1.25 (0.84 - 1.87)	1.04 (0.81 - 1.33)	1.00	0.3325
		MD				
1[a]			1.73 (1.11 - 2.70)	1.14 (0.77 - 1.67)	1.00	0.0642
2[b]			1.66 (1.04 - 2.65)	1.08 (0.72 - 1.60)	1.00	0.1351
3[c]	0.27		1.44 (0.92 - 2.26)	1.04 (0.71 - 1.53)	1.00	0.2572

Adjusted covariates:
[a] Model 1= No adjustment
[b] Model 2 = Age, gender, race and education
[c] Model 3 = Model 2 + Physical activity, self-rated health status, having diabetes, total energy intake, and serum level of vitamin C.
[d] Tertiles cut-off point of FV intake and the number of subjects in each tertile.

Abbreviations – ADL: activities of daily living; IADL: instrumental activities of daily living; MD: movement difficulty; OR: odds ratio; CI: confidence interval; and FV total fruit and vegetable
ORs are odds ratios of limitation in physical function domains (ADL, IADL, and MD) comparing participants in the lower tertiles of fruit and vegetable intake (cup equivalent) to those in the highest tertile.
[*] P-value for trend of the odds of having difficulties in ADL, IADL, and MD across total FV intake tertiles <0.01

4.5 Associations between MD score and dietary and serum carotenoids

Adjusted means for the MD score according to quintiles of serum levels of total carotenoids, unadjusted and adjusted daily dietary intake, and total intake of carotenoids as well as tertiles of FV intake are shown in tables 11 and 12. A greater mean difference of MD score was observed at lower quintiles of carotenoids concentrations compared to the highest quintile; even after controlling for all confounders in model 2 and 3. Based on the pseudo R-squared, almost 50% of the data were explained by the full model. The association between serum carotenoids as a continuous variable and MD score was highly and inversely significant [p for trend = 0.0000 across

all the models] even after controlling for all potential confounders. There was also a similar association between calorie-adjusted dietary intake of carotenoids and MD score although attenuated after controlling for confounders [p for trend = 0.017], but no association was found between absolute dietary and total carotenoids intake and MD score. Also, no associations were observed between FV intake and outcome after adjustment for confounders.

Table 11
Unadjusted and adjusted mean difference of movement difficulty score [a] using regression models (least square mean)

Model	R^2	Main predictors	*(n)*	Movement Difficulty Score Mean difference ±SE					
				1st Quintile	2nd Quintile	3rd Quintile	4th Quintile	5th Quintile [g]	*P* for trend [*]
		Serum carotenoids (ug/dL)	2785						
1[b]				0.642 ±0.07)	0.425 ±0.07	0.281 ±0.06	0.192 ±0.06	1.00	0.0000
2[c]				0.634 ±0.06	0.402 ±0.07	0.276 ±0.06	0.190 ±0.05	1.00	0.0000
3[d]	0.49			0.301 ±0.04	0.163 ±0.06	0.133 ±0.05	0.131 ±0.05	1.00	0.0000
		Dietary carotenoids (mcg/day)	1829						
1[b]				0.371 ±0.08	0.332 ±0.08	0.137 ±0.08	0.190 ±0.08	1.00	0.0000
2[c]				0.251 ±0.08	0.240 ±0.07	0.051 ±0.08	0.133 ±0.08	1.00	0.0037
3[e]	0.33			0.122 ±0.07	0.172 ±0.09	0.033 ±0.08	0.083 ±0.08	1.00	0.0890
		Adjusted dietary carotenoids (mcg/day)	1829						
1[b]				0.231 ±0.07	0.215 ±0.08	0.285 ±0.10	0.230 ±0.08	1.00	0.0000
2[c]				0.232 ±0.07	0.113 ±0.08	0.201 ±0.09	0.170 ±0.09	1.00	0.0013
3[e]	0.34			0.144 ±0.08	0.061 ±0.08	0.185 ±0.08	0.122 ±0.08	1.00	0.0168
		Total intake of carotenoids (mcg/day)	1829						
1[b]				0.390 ±0.08	0.274 ±0.08	0.158 ±0.07	0.178 ±0.08	1.00	0.0000
2[c]				0.261 ±0.08	0.188 ±0.07	0.065 ±0.07	0.116 ±0.09	1.00	0.0064
3[f]	0.31			0.124 ±0.08	0.131 ±0.08	0.063 ±0.08	0.067 ±0.08	1.00	0.1089

[a] MD score: is a score measuring the severity of Movement difficulty, ranges from 2.5 to 10. The higher the score, the higher the severity.
[b] Model 1 = no adjustment.
[c] Model 2 = Age, Gender, Race/Ethnicity and Education.
[d] Model 3 for serum carotenoids = model 2 + body mass index (BMI), physical activity (PA), self-rated health status, told by doctor to have arthritis, and C-reactive protein.
[e] Model 3 for dietary intake of total carotenoids and adjusted intake = model 2 + BMI, PA, and self-rated health status.
[f] Model 3 for total intake (diet + supplement) = model 2 + BMI, PA, and self-rated health status.
[g] Quintiles cut-off points of serum carotenoids, dietary intake, adjusted dietary intake, and total carotenoids used from previous tables

[*] P-value for trend of mean difference of MD score across main predictors of (serum carotenoids, dietary intake of total carotenoids, adjusted dietary intake of total carotenoids, and total intake of total carotenoids) quintiles <0.01.

According to FV intake, participants in the lower tertiles have greater differences in MD scores compared to those in the highest tertile. The trends of having smaller mean differences of MD scores across increasing FV intake tertiles were statistically significant even after adjusting for covariates in model 2, [P = 0.0026]. However, after further controlling for confounders in model 3 the P for trend was no longer significant [table 12].

Table 12
Unadjusted and adjusted mean difference of MD score and FV intake using regression models (least square mean)

Model	R^2	Main predictor	*(n)*	Movement Difficulty Score Mean difference ±SE			
				1st Tertile (≤ 1.65 cup) n=581	2nd Tertile (1.66 - ≤ 3.043 cup) n=581	3rd Tertile [d] (> 3.043 cup) n=596	*P* for trend [*]
		Total FV intake (cup equivalent)	1758				
1[a]				0.368 ±0.07	0.216 ±0.09	1.00	0.0001
2[b]				0.262 ±0.07	0.154 ±0.09	1.00	0.0026
3[c]	0.48			0.101 ±0.06	0.064 ±0.06	1.00	0.0882

Note: MD score: is a score to measure the severity of Movement difficulty. The low the score the, the least sever the problem.
In all models lowest quintile or tertile were compared to the highest.
[a] Model 1 = no adjustment.
[b] Model 2 = Age, Gender, Race/Ethnicity and Education.
[c] Model 3 for total fruit and vegetable intake = model 2 + PA, self-rated health status, told by doctor to have diabetes, serum vitamin C, and total energy intake.
[d] Tertiles cut-off point of FV intake and the number of subjects in each tertile.

Abbreviation - MD: Movement difficulty; and FV: fruit and vegetable.
[*] P-value for trend of the mean difference of MD score across total FV intake tertiles <0.01.

According to the full regression models used in this analysis, the measurement of goodness-of-fit, using Wald-F test showed that the overall models are all significant (P-value < 0.01), and a good fit for the data.

Chapter 5: Discussion and Conclusion

5.1 Discussion

This study examined the associations between serum carotenoids, FV, total and dietary intake of carotenoids, and physical function in U.S. older adults. Our results show an inverse association between serum concentrations of carotenoids and limitations in PF domains of ADL, IADL, and MD. As carotenoids concentration increase across the quintiles, the odds of having limitation in ADL, IADL, and MD decrease. Additionally, a significant inverse association was also found between absolute and relative dietary intake of carotenoids and limitation in IADL, even after including intake from supplement and adjusting for confounders. Our findings also show an association between lower daily dietary intakes of carotenoids and limitation in IADL even after adjustment for total energy intake and several confounders, but no associations with other PF domains. These results are not consistent with results of the InCHIANTI study, which showed that higher intake of β-carotene was associated with greater skeletal muscle strength (Cesari et al., 2004).

The results of the present study are consistent with findings from the prospective InCHIANTI study in which total plasma carotenoids at enrollment were independent predictors of a decline in walking speed among older community-dwelling men and women. In addition, participants with low plasma carotenoids concentration experienced a greater decline in hip, knee, and grip muscle strength over a period of 6 years compared to persons with high plasma carotenoids (Lauretani et al., 2008). Another longitudinal study also reported an association between low total carotenoids concentration, a marker for FV intake, and low walking speed, suggesting that carotenoids may be protective against a decline in walking speed in older women during three years of follow-up (Alipanah et al., 2009).

The InCHIANTI study also showed strong positive associations between plasma concentrations of α- and γ- tocopherols and physical performance (Cesari et al., 2004). Carotenoids are lipid-soluble antioxidants that work synergistically with vitamin E. In another study, low selenium levels were found to be associated with low walking speed (Alipanah et al., 2009). Therefore, it may be possible that the admixture of several antioxidants are essential to maintaining healthy PF. Additionally, since FV are rich sources of antioxidants, including the carotenoids, vitamin C, flavonoids, and other polyphenols, it cannot be determined from our study whether serum carotenoids play a direct role in maintaining PF or if other components in FV interact with carotenoids to contribute to healthier PF. In addition, due to many factors affecting the bioavailability, absorption, metabolism, or storage of carotenoids, the exact mechanisms of their effects on physical performance are still far from being fully understood.

Since the correlation between dietary and serum carotenoids was relatively weak, it may be possible that serum carotenoid is a biomarker for FV intake generally. However, in our study, even FV intake was not associated with PF. These results are inconsistent with findings from previous longitudinal studies in which high FV intake was shown to protect against decline in PF (Houston et al., 2005; Tomey et al., 2008). Other studies that examined individual nutrients show that there is no association between intakes of vitamins C and E and physical performance (Clarkson, 1995; Maxwell, 1995). However, intervention studies showed improvements in recovery from exercise with higher antioxidant intake which included β-carotene α-tocopherol, ascorbic acid, selenium and, glutathione (Clarkson & Thompson, 2000). In our analysis, several serum vitamin measures such as vitamin C, the first antioxidant line of defense, and vitamin E, a major lipid-soluble antioxidant that prevents lipid peroxidation in lipoproteins (Clarkson & Thompson, 2000), were initially included in the regression models as potential confounders, but they were not significantly associated with PF

domains and were taken out of the regression models. Thus, the relationship between antioxidant intake, physical performance, and muscular strength is complex and studies show inconsistent results for dietary intake. Moreover, the benefits of taking carotenoid supplements remains uncertain and controversial due mainly to the results of an intervention study that showed an increase in lung cancer among smokers who took carotenoid supplements compared to those who did not take those supplements (Goralczyk, 2009). More mechanistic studies are needed to understand the function of carotenoids and other antioxidants on PF. Further studies may also be needed to study the association between carotenoids and cognitive function. In our study, dietary intake of carotenoids was associated only with IADLs, which involves more cognitive function than skeletal muscle strength.

We also examined the association between total intake of carotenoids from diet and supplements and PF but the sample size was small [n=249], and the association was not significant. Our findings were consistent with an intervention study of individuals aged 65-85 years that showed increased FV consumption has no significant effect on measures of lower-extremity PF, but modestly increases grip strength (Neville et al., 2013). Possible explanations for the insignificant associations of FV intake and PF domains may be that older adults improve their eating habits with age to include more FV, diluting the life-long impact of FV on PFL.

To our knowledge, this is the first study that scored the MD questions as suggested by national Center for health statistics and examined the score in association with carotenoids. Our findings showed that the MD score was highly associated with serum concentration of carotenoids, and may be a good indicator of PF status.

Participants of our study with no PFL tended to be younger, with higher educational attainment and self-rated their health status as excellent to good. Participants who reported

difficulty in one or more of the PF domains, compared to those with no difficulty, tended to be overweight or obese, physically inactive, and did not attain the sufficient MET score as suggested by the American guidelines for physical activity. Individuals with PFL may be restricted and unable to be physically active, which consequently leads to weight gain. Modifiable risk factors, such as greater physical activity and prevention of obesity, may protect against the decline in PF.

One limitation of this study is that the cross-sectional design does not allow for the determination of cause-and-effect relationship between carotenoids and physical performance. Therefore, more longitudinal and experimental studies are needed to understand mechanistically the role and the association between carotenoids and PF. Additionally, dietary intake was self-reported and is subject to reporting bias which may attenuate associations. However, serum carotenoids is an objective biomarker and shown to be strongly associated with functional limitation, but may not reflect only dietary carotenoids. Also, PF status was self-reported, which may bias the results.

The strength of this study is that dietary and serum carotenoids and FV intake were available and could be examined in association with multiple measures of PF in older adults. Previous studies examined either total serum carotenoids (Alipanah et al., 2009; Lauretani et al., 2008) or only dietary intake of β-carotene (Cesari et al., 2004) on physical performance. Other studies focused only on assessing the association between FV intake and PF in younger adults and from smaller samples (Houston, Stevens, Cai, & Haines, 2005; Tomey et al., 2008). The only intervention study examined the effect of increased FV consumption on grip strength and lower-extremity physical function (Neville et al., 2013). Another strength of this study is that the data is derived from a nationally representative sample of the U.S. older adult population. The NHANES physical function questionnaire is comprehensive and includes information on ADL, IADL, and physical function of lower and upper body function.

Finally, due to the wide array of covariates assessed in NHANES and the large sample size, many of the potential confounders were collected and controlled for in the analysis.

5.2 Conclusion

In conclusion, this study demonstrates an inverse association between serum carotenoids and PF in older adults in the US. Also, higher dietary intake of total carotenoids was inversely associated with IADL limitation. Low fruits and vegetables intakes tended to be associated with a higher MD score and were independently associated with IADL limitation. Similar studies were conducted previously, but the results are inconsistent.

Our study found that serum carotenoids have a stronger inverse association with PF domains than dietary intake, total intake, or FV consumption. There are several possible explanations for these findings: the dietary intake estimation of carotenoids using one day of 24-hour recall may not be sufficient to capture the usual intake of FV. In addition, the subjective nature of self-reported dietary intake assessment methods may affect the accuracy of the exact dietary intake. This limitation can be compensated for by the use of serum biomarkers, which provide an objective measure of dietary consumption without the bias of self-reported dietary intake errors. In our study, serum carotenoids concentrations as biomarkers of FV intakes may reflect the long-term intake and ability to detect changes in intake over time.

It is currently accepted that FV are healthy food groups composed of a complex mix of polyphenols, antioxidants, and vitamins that prevent or delay the onset of chronic conditions through different mechanisms. More experimental studies are needed to identify how mechanistically carotenoids function intramuscularly. It is not possible through epidemiological studies to tease out the associations of different compounds due to the difficulties in controlling for known and unknown confounders, and that is why it is more efficient to look at food patterns

instead of individual nutrients. Such an approach is currently common practice. For example, short-term randomized trials using Dietary Approaches to Stop Hypertension Studies [DASH] (Appel et al., 1997) and Mediterranean-style diet were shown to reduce cardiovascular risk factors (Estruch et al., 2006; Estruch et al., 2013). Although a Mediterranean-style diet that is high in FV has shown a protective effect against chronic diseases, further studies are needed to examine whether a similarly healthy diet can protect against losing muscle mass and strength, decline in physical performance and progression to disability among older adults.

Studies are needed to identify and address the barriers that older adults may face in obtaining and consuming FV. In fact, retarding the decline in PF through modifiable risk factors, such as FV intake, protein intake, and physical activity, may contribute to maintaining independence longer. Moreover, due to non-availability of studies using the MD score to evaluate PF, more observational studies are needed to assess and examine the association of MD score with PF. A comparison between MD scores and physical performance test scores could be conducted in future studies to evaluate how each measurement correlates with PFL.

Several future research directions seem important to pursue, including: the measurement of free radical production in skeletal muscle at diverse ages; increasing FV intakes; and determining the influence of increasing FV intake on reducing oxidative stress in specific skeletal muscles. Also, it is essential to identify mechanistically the compounds that are associated with PF so that a better understanding could lead to interventions.

Finally, serum carotenoids appear to be a good marker of PFL. Decline in PF is strongly related to loss of muscle mass and strength (Janssen, Heymsfield, & Ross, 2002). Oxidative stress and increased oxidative damage to DNA, protein, and lipids that occurs with aging in skeletal muscle are associated with atrophy and loss of muscle fibers (Lobo, Patil, Phatak, & Chandra, 2010; Morgan & Partridge, 2003;

Trifunovic & Larsson, 2008). Serum carotenoids may play an important role in reducing oxidative stress through the quenching of ROS (Tanumihardjo, 2012).

Appendix

Table 3
Characteristics of the study participants by gender and physical function domains

Gender	Characteristics	Physical Function Domains								
		(n)	ADL (n=2904)[a]		(n)	IADL (n=2888)[a]		(n)	MD (n=2905)[a]	
			Not-limited [b]	*Limited* [b]		*Not-limited* [b]	*Limited* [b]		*Not-limited* [b]	*Limited* [b]
Men	(n/%)	1469	1110 (75.6)	359 (24.4)	1457	1071 (73.5)	386 (26.5)	1469	593 (40.4)	876 (59.6)
	Socio-demographic									
	Age (y) (mean ±SE)		70.1 ±0.27	71.9 ±0.64		69.9 ±0.28	72.0 ±0.52		68.8 ±0.30	71.7 ±0.31
	Race/ethnicity (n/%)	1469			1457			1469		
	Non-Hispanic white		693 (62.4)	212 (59.1)		669 (62.5)	228 (59.0)		352 (59.4)	553 (63.1)
	Non-Hispanic black		182 (16.4)	65 (18.1)		172 (16.0)	72 (18.7)		102 (17.2)	145 (16.6)
	Mexican American		235 (21.2)	82 (22.8)		230 (21.5)	86 (22.3)		139 (23.4)	178 (20.3)
	Education (n/%)	1469			1457			1469		
	Less than high school		384 (34.6)	168 (46.8)		361 (33.7)	185 (47.9)		192 (32.4)	360 (41.1)
	Completed high school		259 (23.3)	77 (21.5)		248 (23.2)	85 (22.1)		132 (22.2)	204 (23.3)
	Some College and above		467 (42.1)	114 (31.8)		462 (43.1)	116 (30.0)		269 (45.4)	312 (35.6)
	Lifestyle factors									
	Smoking status (n/%)	1469			1457			1469		
	Not Smoker		359 (23.3)	103 (28.7)		353 (33.0)	107 (27.7)		203 (34.2)	259 (29.6)
	Current Smoker		579 (52.2)	205 (57.1)		555 (51.8)	219 (56.8)		297 (50.1)	487 (55.6)
	Former Smoker		172 (15.5)	51 (14.2)		163 (15.2)	60 (15.5)		93 (15.7)	130 (14.8)
	Physical activity (n/%) [c]	1469			1457			1469		
	Inactive		466 (42.0)	245 (68.2)		452 (42.2)	249 (64.5)		220 (37.1)	491 (56.1)
	Insufficient		94 (8.50)	29 (8.10)		87 (8.12)	35 (9.10)		47 (8.00)	76 (8.70)
	Sufficient		550 (49.5)	85 (23.7)		532 (49.7)	102 (26.4)		326 (54.9)	309 (35.3)
	Alcohol consumption (n/%)	1421			1411			1421		
	None drinker		229 (21.2)	80 (23.3)		220 (21.1)	86 (23.2)		117 (20.2)	192 (22.8)
	Moderate drinker		675 (62.6)	223 (65.0)		654 (62.8)	237 (64.1)		365 (62.9)	533 (63.4)
	Excessive drinker		174 (16.2)	40 (11.7)		167 (16.1)	47 (12.7)		98 (16.9)	116 (13.8)
	Self-rate health status (n/%)	1422			1412			1422		
	Good health		856 (79.3)	180 (52.5)		842 (80.8)	189 (51.0)		494 (85.2)	542 (64.4)
	Poor health		223 (20.7)	163 (47.5)		200 (19.2)	181 (49.0)		86 (14.8)	300 (35.6)
	Told by Dr. to have … (n/%)									
	Cardiovascular disease	1469	838 (74.0)	293 (26.0)	1457	789 (70.5)	330 (29.5)	1469	420 (37.2)	711 (62.8)
	Diabetes	1469	218 (66.0)	112 (34.0)	1457	193 (59.2)	133 (40.8)	1469	91 (27.6)	239 (72.4)
	Cancer	1469	220 (78.3)	61 (21.7)	1457	203 (73.0)	75 (27.0)	1469	101 (36.0)	180 (64.0)
	Arthritis	1469	389 (63.3)	226 (36.7)	1457	391 (64.0)	220 (36.0)	1469	150 (24.4)	465 (75.6)
	Osteoporosis		30 (55.6)	24 (44.4)		26 (50.0)	26 (50.0)		7 (13.0)	47 (87.0)
Women	(n/%)	1436	1052 (73.3)	383 (26.7)	1431	854 (59.7)	577 (40.3)	1436	396 (27.6)	1040 (72.4)
	Socio-demographic									
	Age (y) (mean ±SE)		70.7 ±0.27	73.1 ±0.50		70.3 ±0.30	72.9 ±0.37		69.4 ±0.50	72.1 ±0.34
	Race/ethnicity (n/%)	1435			1431			1436		
	Non-Hispanic white		663 (63.0)	201 (52.5)		521 (61.0)	340 (58.9)		236 (59.6)	628 (60.4)
	Non-Hispanic black		162 (15.4)	77 (20.1)		128 (15.0)	111 (19.2)		63 (15.9)	176 (16.9)
	Mexican American		227 (21.6)	105 (27.4)		205 (24.0)	126 (21.8)		97 (24.5)	236 (22.7)
	Education (n/%)	1435			1431			1436		
	Less than high school		352 (33.4)	185 (48.3)		280 (32.8)	255 (44.1)		141 (35.6)	396 (38.1)
	Completed high school		308 (29.3)	103 (26.9)		251 (29.4)	159 (27.6)		112 (28.3)	300 (28.8)
	Some College and above		392 (37.3)	95 (24.8)		323 (37.8)	163 (28.3)		143 (36.1)	344 (33.1)
	Lifestyle factors									
	Smoking status (n/%)	1435			1431			1436		
	Not Smoker		606 (57.6)	231 (60.3)		495 (58.0)	338 (58.6)		246 (62.1)	592 (56.9)
	Current Smoker		334 (31.8)	114 (29.8)		269 (31.5)	179 (31.0)		110 (27.8)	338 (32.5)
	Former Smoker		112 (10.7)	38 (9.90)		90 (10.5)	60 (10.4)		40 (10.1)	110 (10.6)
	Physical activity (n/%) [c]	1435			1431			1436		
	Inactive		500 (47.5)	265 (69.2)		374 (43.8)	388 (67.2)		162 (40.9)	604 (58.1)
	Insufficient		140 (13.3)	34 (8.90)		104 (12.2)	69 (12.0)		51 (12.9)	123 (11.8)
	Sufficient		412 (39.2)	84 (21.9)		376 (44.0)	120 (20.8)		183 (46.2)	313 (30.1)
	Alcohol consumption (n/%)	1384			1380			1384		
	None drinker		522 (51.0)	204 (56.7)		422 (50.7)	301 (55.0)		197 (51.2)	529 (53.0)
	Moderate drinker		371 (36.2)	124 (34.4)		301 (36.2)	193 (35.0)		130 (33.8)	365 (36.5)
	Excessive drinker		131 (12.8)	32 (8.90)		109 (13.1)	54 (10.0)		58 (15.0)	105 (10.5)
	Self-rate health status (n/%)	1386			1382			1386		
	Good health		782 (76.3)	157 (43.5)		657 (79.0)	281 (51.0)		324 (84.2)	615 (61.4)
	Poor health		243 (23.7)	204 (56.5)		175 (21.0)	269 (49.0)		61 (15.8)	386 (38.6)
	Told by Dr. to have … (n/%)									
	Cardiovascular disease	1435	797 (70.2)	338 (29.8)	1431	628 (55.5)	503 (44.5)	1436	273 (24.0)	863 (76.0)
	Diabetes	1435	175 (55.0)	144 (45.0)	1431	133 (41.8)	185 (58.2)	1436	53 (16.6)	267 (83.4)
	Cancer	1435	177 (70.0)	76 (30.0)	1431	142 (56.0)	111 (44.0)	1436	60 (23.7)	193 (76.3)
	Arthritis	1435	541 (64.0)	305 (36.0)	1431	414 (49.0)	429 (51.0)	1436	137 (16.2)	710 (83.8)
	Osteoporosis	1435	250 (70.2)	106 (29.8)	1431	181 (51.0)	173 (49.0)	1436	71 (20.0)	285 (80.0)

[a] (n) is the total study population under each of the physical function domain .
[b] Not-limited: If participant reported no difficulty in all of the questions within the ADL, IADL, or MD domains.
Limited: If participant reported some difficulty in one or more of the questions within the ADL, IADL, or MD domains.
[c] Physical activity: calculated using metabolic equivalent of task (MET) minutes per week as suggested by physical activity guidelines for Americans. Defined as inactive if participant has 0 MET-minutes per week; "insufficient" to meet the guidelines if participant has 1 to 499 MET-minutes per week; and "sufficient" to meet the guidelines if participant has 500 and more
MET-minutes per week.
Abbreviation- ADL: Activities of daily living; IADL: instrumental activities of daily living; and MD: movement difficulty.

Table 4

Dietary intakes and anthropometrics of the study participants by gender and physical function domains

Gender	Characteristics	Physical Function Domains								
		(n)	ADL (n=2904)[a]		(n)	IADL (n=2888)[a]		(n)	MD (n=2905)[a]	
			Not-limited [b]	*Limited* [b]		*Not-limited* [b]	*Limited* [b]		*Not-limited* [b]	*Limited* [b]
Men	MD Score [c d]	1469	2.60 ±0.04	3.99 ±0.11	1457	2.54 ±0.02	3.86 ±0.08	1469	2.50 ±0.01	3.24 ±0.06
	Dietary intakes									
	FV intakes (cup)(mean ±SE)	866	2.90 ±0.13	2.78 ±0.16	861	2.88 ±0.14	2.82 ±0.17	866	3.09 ±0.14	2.72 ±0.13
	Total energy intake (kcal/day)(mean ±SE)	1469	2096 ±35.5	1938 ±48.5	1457	2098 ±33.9	1956 ±50.0	1469	2088 ±46.2	2042±36.6
	Protein intake (gm/day)(mean ±SE)	1469	81.6 ±1.60	74.1 ±1.90	1457	81.7 ±1.50	74.6 ±2.43	1469	81.7 ±1.50	74.6 ±2.43
	Fat intake (gm/day)(mean ±SE)	1469	80.9 ±1.75	77 ±3.00	1457	81.0 ±1.71	77.8 ±2.62	1469	79.5 ±2.22	80.5 ±1.90
	Dietary Carotenoids intake (mcg/day)[c]	904	7792 ±480	9264 ±1339	899	8605 ±694	6563 ±716	904	8567 ±1010	7699 ±703
	Adjusted dietary carotenoids intake (mcg/day)[c]	904	7379 ±39.0	8786 ±1349	899	7826 ±386	6180 ±759	904	8195 ±519	6813 ±498
	Anthropometrics									
	Height (cm)(mean ±SE)	1438	174.6 ±0.28	173 ±0.55	1426	174.5 ±0.26	174 ±0.53	1438	174 ±0.46	174.5 ±0.25
	Weight (kg)(mean ±SE)	1443	86.3 ±0.55	88.5 ±1.80	1433	86.5 ±0.55	87.7 ±1.35	1443	83.7 ±0.84	89.1 ±0.71
	BMI (kg/m2) (n/%)	1434	28.3 ±0.15	29.5 ±0.56	1424	28.4 ±0.15	29 ±0.40	1434	27.6 ±0.21	29.3 ±0.23
	<18.5 underweight		12 (1.10)	7 (2.10)		9 (0.85)	10 (2.70)		8 (1.36)	11 (1.30)
	18.5-24.9 normal		275 (25.0)	91 (27.3)		261 (24.7)	103 (28.2)		165 (28.0)	201 (23.8)
	25-29.9 overweight		502 (45.6)	123 (36.8)		491 (46.4)	129 (35.3)		282 (47.8)	343 (40.6)
	30+ Obese		311 (28.3)	113 (33.8)		298 (28.1)	123 (33.7)		135 (22.9)	289 (34.3)
	Lean body mass (kg)(mean ±SE)	1393	46.2 ±0.35	45.1 ±0.88	1373	46.5 ±0.34	43.6 ±0.10	1393	46.2 ±0.58	45.7 ±0.49
Women	MD Score [c d]	1435	2.71 ±0.03	4.38 ±0.07	1431	2.56 ±0.02	4.11 ±0.06	1436	2.50 ±0.01	3.54 ±0.06
	Dietary intakes									
	FV intakes (cup)(mean ±SE)	891	2.75 ±0.09	2.44 ±0.16	887	2.89 ±0.10	2.35 ±0.10	892	2.95 ±0.16	2.56 ±0.09
	Total energy intake (kcal/day)(mean ±SE)	1435	1607 ±17.3	1492 ±35.1	1431	1632 ±19.8	1497 ±24.3	1436	1642 ±31.7	1555 ±19.2
	Protein intake (gm/day)(mean ±SE)	1435	62.3 ±1.20	57.5 ±1.41	1431	63.7 ±1.24	57.2 ±1.20	1436	63.7 ±1.24	57.2 ±1.20
	Fat intake (gm/day)(mean ±SE)	1435	62 ±0.88	57 ±1.91	1431	63 ±0.95	59 ±1.70	1436	64 ±1.66	60 ±1.15
	Dietary Carotenoids intake (mcg)[c]	924	8119 ±646	6060 ±609	922	7946 ±679	6214 ±712	925	8205 ±735	6883 ±537
	Adjusted dietary carotenoids intake [c]	924	8510 ±544	7881 ±565	922	8384 ±554	8382 ±560	925	8623 ±611	8289 ±484
	Anthropometrics									
	Height (cm) (mean ±SE)	1397	160.3 ±0.27	159.2 ±0.47	1394	160.5 ±0.32	159.2 ±0.41	1397	161 ±0.50	159.6 ±0.30
	Weight (kg) (mean ±SE)	1409	71.6 ±0.56	77.2 ± 1.28	1405	70.9 ±0.60	76.2 ±1.06	1409	69.2 ±0.58	74.4 ±0.74
	BMI (kg/m2) (n/%)	1397	27.8 ±0.21	30.4 ±0.40	1394	27.5 ±0.22	29.9 ±0.35	1397	26.6 ±0.16	29.2 ±0.26
	<18.5 underweight		12 (1.20)	2 (0.70)		10 (1.20)	4 (0.73)		5 (1.30)	9 (0.90)
	18.5-24.9 normal		330 (31.7)	76 (21.4)		280 (33.1)	125 (22.8)		140 (35.8)	266 (26.4)
	25-29.9 overweight		360 (34.6)	111 (31.2)		292 (34.6)	178 (32.4)		146 (37.3)	325 (32.3)
	30+ Obese		339 (32.6)	167 (47.0)		263 (31.1)	242 (44.1)		100 (25.6)	406 (40.4)
	Lean body mass (kg)(mean ±SE)	1373	46.7 ±0.48	46.3 ±0.12	1369	46.9 ±0.40	46.1 ±0.11	1374	47.9 ±0.71	46.1 ±0.69

[a] (n) is the total study population under each of the PF domain.

[b] Not-limited: If participant reported no difficulty in all of the questions within the ADL, IADL, or MD domains.
Limited: If participant reported some difficulty in one or more of the questions within the ADL, IADL, or MD domains.

[c] Values are median ± standard error (SE)

[d] MD score: is a score to measure the severity of Movement difficulty. The low the score the, the least sever the problem.

Abbreviation- ADL: activities of daily living; IADL: instrumental activities of daily living; MD: movement difficulty; FV: fruits and vegetables; and BMI: body mass index.

Table 5
Serum measurements of the study participants by gender and PF domain

Gender	Characteristics	Physical Function Domains								
		(n)	ADL (n=2904)[a]		(n)	IADL (n=2888)[a]		(n)	MD (n=2905)[a]	
			Not-limited [b]	*Limited* [b]		*Not-limited* [b]	*Limited* [b]		*Not-limited* [b]	*Limited* [b]
	Serum measurement									
Men	Total cholesterol (mmol/L)	1469	5.00 ± 0.03	4.95 ±0.09	1457	5.00 ±0.03	4.95 ±0.07	1469	5.07 ±0.04	4.91 ±0.05
	HDL cholesterol (mmol/L)	1469	1.29 ±0.01	1.26 ±0.02	1457	1.29 ±0.01	1.25 ±0.02	1469	1.32 ±0.02	1.25 ±0.01
	LDL cholesterol (mmol/L)	1460	2.92 ±0.03	2.90 ± 0.08	1421	2.91 ±0.03	2.92 ±0.07	1460	3.00 ±0.05	2.84 ±0.04
	Triacylglycerol (mmol/L) [c]	1460	1.40 ±0.04	1.45 ±0.09	1448	1.42 ±0.04	1.41 ± 0.06	1460	1.37 ±0.07	1.44 ±0.04
	Uric Acid (mg/dL)	1461	6.10 ±0.05	6.16 ±0.13	1449	6.11 ±0.05	6.11 ±0.09	1461	6.02 ±0.07	6.17 ±0.06
	C-reactive protein (mg/dL) [c]	1469	0.20 ±0.01	0.30 ±0.03	1457	0.20 ±0.01	0.31 ±0.03	1469	0.17 ±0.01	0.25 ±0.01
	Total Plasma carotenoids (ug/dL)	1402	88.7 ±2.20	75.2 ±2.43	1392	88.4 ±2.22	77.1 ±2.86	1402	94.1 ±3.31	79.5 ±1.59
	Vitamin D (ng/mL)	1469	24.4 ±0.42	22.2 ±0.62	1457	24.3 ±0.43	22.7 ±0.67	1469	25.3 ±0.52	22.9 ±0.48
	Vitamin C (mg/dL)	1456	1.04 ±0.02	0.19 ±0.03	1444	1.04 ±0.02	0.93 ±0.03	1456	1.06 ±0.02	0.98 ±0.03
	Vitamin E (mg/dL)	1469	1565 ±31.0	1421 ±54.2	1457	1569 ±33.5	1422 ±48.1	1469	1564 ±33	1512 ±39.5
Women	Total cholesterol (mmol/L)	1434	5.60 ±0.04	5.22 ±0.05	1430	5.62 ±0.04	5.32 ±0.05	1435	5.66 ±0.07	5.44 ±0.04
	HDL cholesterol (mmol/L)	1433	1.60 ±0.02	1.51 ±0.02	1429	1.62 ±0.02	1.52 ±0.02	1434	1.66 ±0.02	1.55 ±0.02
	LDL cholesterol (mmol/L)	1430	3.23 ±0.04	2.92 ±0.04	1426	3.25 ±0.03	3.00 ±0.05	1431	3.29 ±0.05	3.10 ±0.04
	Triacylglycerol (mmol/L) [c]	1432	1.43 ±0.04	1.52 ±0.06	1428	1.43 ±0.05	1.48 ± 0.06	1433	1.31 ±0.05	1.53 ±0.04
	Uric Acid (mg/dL)	1432	5.18 ±0.05	5.63 ±0.12	1428	5.07 ±0.05	5.63 ±0.08	1433	4.91 ±0.06	5.44 ±0.06
	C-reactive protein (mg/dL) [c]	1435	0.24 ±0.01	0.32 ±0.04	1430	0.22 ±0.01	0.32 ±0.03	1435	0.19 ±0.01	0.28 ±0.02
	Total Plasma carotenoids (ug/dL)	1382	102.7 ±2.65	79.9 ±3.26	1378	106.1 ±2.93	83.6 ±3.03	1383	111.1 ±3.67	92.0 ±2.82
	Vitamin D (ng/mL)	1435	23.8 ±0.34	21.1 ±0.61	1431	24.4 ±0.44	21.1 ±0.50	1436	25.3 ±0.52	22.2 ±0.38
	Vitamin C (mg/dL)	1425	1.23 ±0.02	1.08 ±0.04	1421	1.22 ±0.02	1.14 ±0.03	1426	1.24 ±0.03	1.17 ±0.02
	Vitamin E (mg/dL)	1435	1776 ±25.7	1607 ±53.3	1431	1784 ±25.7	1657 ±38.8	1436	1744 ±26.3	1735 ±34.4

[a] (n) is the total study population under each of the PF domain.
[b] Not-limited: If participant reported no difficulty in all of the questions within the ADL, IADL, or MD domains.
Limited: If participant reported some difficulty in one or more of the questions within the ADL, IADL, or MD domains.
Values are means (SE) or [c] values are median ± standard error (SE).

Abbreviation- ADL: activity of daily living; IADL: instrumental activity of daily living; MD: Movement difficulty; HDL: high density lipoprotein; and LDL: low density lipoprotein.

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