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The relationship between habitual dietary protein intake and dual task performance in sedentary, recreationally active, and masters athlete older adults

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Abstract

As the body ages, physical and cognitive declines can result in balance and mobility deficits, but research has shown that proper nutrition and exercise can help maintain physical and mental capacity. The purpose of this study was to analyze the relationship between habitual dietary protein intake and dual task performance in sedentary (SED), recreationally active (RA), and masters athletes (MA). To measure physical activity levels, the Rapid Physical Activity Questionnaire (RAPA) was completed by all participants. The participants were placed into a high or low protein group using the ASA-24 hour dietary recall. If the participant consumed less than 0.8 g/kg of protein per day, they were placed in the low protein group; if the participant consumed more than 0.8 g/kg of protein per day, they were placed in the high protein group. Participants completed four different walking tasks: habitual speed, maximal speed, dual-task habitual speed, and dual-task maximal speed. Gait speed was measured over a distance of 10 meters. SED, RA, and MA consumed a mean of 0.84, 1.13, and 1.57 grams of protein per kilogram body weight per day, respectively. MA consumed significantly more protein than SED or RA participants ($\alpha < .05$). The low protein group consumed 0.84 g/kg of protein \pm 0.39 while the high protein group consumed 1.30 g/kg of protein \pm 0.50. There was no significant correlation between amount of protein consumed and dual task performance. While the results were for dual task performance not statistically significant, they may have clinical significance; when comparing the high and low protein groups for the dual task habitual trial, the high protein group covered the 10-m distance 0.73 seconds faster than the low protein group. Clinically, the higher protein group may be able to perform activities of daily living more efficiently.

Keywords: Dual task, protein, gait, masters athlete

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Exercise, cognitive function, and diet are imperative topics in the health of older adults. The physical, cognitive, and nutritional demands of the body become a central focus to maintain a healthy life. As the body ages, physical and cognitive declines can result in balance and mobility deficits. Some decline is inevitable with age, but research has shown that proper nutrition and exercise can help maintain physical and mental capacity.

Dual tasking is defined as performing two tasks simultaneously. The speed of dual task processing is directly related to executive function of the brain. When analyzing executive function of the brain, dual task processing is faster when functional activation in the brain is higher (Nagamatsu et al., 2016). Compared with younger adults, there is a smaller volume of white matter in the brain of older adults resulting in slower cellular signaling. Brain atrophy among older adults is associated with decreased dual task walking speed (Doi et al., 2011). In healthy adults, a decline in executive function can begin as early as 30 years old; therefore, executive function and dual task are often impaired by late age (Wong et al., 2008). Along with executive function, balance and mobility also decline with age putting older adults at an increased risk for falling. Dual task has been shown to directly relate to fall risk. Individuals with faster dual task processing are at a lower fall risk than individuals with slow dual task processing (Nagamatsu et al., 2016). Studies have shown that dual task can be improved by gait and cognitive training. Exercises used to train dual task are task analysis and postural control exercises (Plummer-D'Amato, 2012). While training the body improves dual task, little research has been done to show the relationship between habitual dietary protein intake and dual task

performance in older adults. Exercise training is beneficial, but not all older adults are able to exercise and perform physical activity; therefore, alternatives for improving dual task are warranted.

Dietary protein intake has been a controversial topic in gerontology. The Recommended Dietary Allowance (RDA) for protein is 0.8-1.2 g/kg of body weight per day. Meeting the minimum RDA standard for protein consumption has been linked to improved physical performance and muscle mass (Filion et al., 2012). Dynapenia, the decrease in muscular strength with age, is directly related to dietary protein intake among older adults (Filion et al., 2012). A higher protein intake during caloric restriction maintains muscle relative to weight lost, which in turn enhances physical function in older women (Mojtahedi et al., 2011). Since greater physical performance is a factor in dual task, protein intake could also play a role in an older adult's dual task ability.

Exercise participation among older adults improves fall risk, physical performance, and cardiovascular fitness. In older adults, regular exercise and acute exercise programs increase gait speed and cognition (Albinet et al., 2014). The cardiorespiratory fitness hypothesis states that improving aerobic fitness or increasing VO₂ max increases cerebral blood flow that ultimately leads to more oxygen and better executive function in the brain (Albinet et al. 2014). Previous study results reveal that older women with high fitness levels showed better executive function; there was more oxygen and thus more activation of the dorsolateral prefrontal cortex (Albinet et al. 2014). The dorsolateral prefrontal cortex plays an important role in executive functions such as working memory, abstract reasoning, and planning. With regard to muscular strength, Choquette and colleagues (2013) found that aerobic and resistance training at higher intensities for six months improved muscle strength and function in sedentary post-menopausal women.

Exercise is closely related to strength and muscle mass, and exercise can be influenced by diet; furthermore, exercise impacts dual task, but it is unknown if there is also a correlation between dietary protein intake and dual task processing among different physical activity levels.

When comparing three different activity levels, sedentary, recreationally active, and masters athletes, the masters athletes show greater maximal gait speed and maximal speed dual tasking than both the sedentary and recreationally active groups; however no differences for dual task exist between sedentary and recreationally active groups. (Glenn et al., 2015). Glenn et al. (2015) believe that the sport-based activities the masters athlete engage in may have more benefits than total physical activity accumulation because they require elements of physical fitness and mental awareness. For both single and dual task habitual trials, no differences exist between groups. Glenn et al. (2015) were the first to report on gait speed in masters athlete and suggested a competitive lifestyle may be beneficial even at late middle age.

Masters athletes are a relatively understudied population with increased attention in literature in recent years. Power et al. (2016) have shown that masters athletes have 14% more excitable muscle mass and 28% more functioning motor units than age-matched controls. Masters athletes also have greater neuromuscular control. The study shows that masters athletes have a decrease in the amount of motor units lost that is associated with aging (Power et al., 2016). Glenn et al. (2016) also show that masters athletes exhibit better peak power and peak velocity than both sedentary or recreationally active older adults; however, there were no differences in average velocity or average power. The study suggests that maintaining a competitive lifestyle with age improves lower body functional power (Glenn et al., 2016). Fear of falling is often accepted as a predominant reason why older adults choose do not want to engage in strength and balance exercises. Yardley et al. (2007) show that an older adults' choice to engage in strength and balance training is determined more by coping appraisal than threat appraisal. The study showing fear of falling played less of a role than coping appraisal. Coping appraisal was determined by enjoyment of the activity, mood, confidence, and believing exercise training was appropriate for someone like them (Yardley et al., 2007). Yardley et al. (2007) also demonstrate that older adults' intent to carry out exercise was based on the opinions of whether their family, friends, and their doctor think strength and balance training is suitable for them. Because some older adults do not have the desire to carry out programs that could reduce fall risk, and some may not be able to perform exercise due to disease or other limitations, other alternatives are necessary.

Many studies have examined diet and dual task separately as it relates to older adults, but no study has explored the relationship between the two entities. Because some people are not able to perform physical activity and exercise to improve dual task as they age, alternatives are needed; this study explores the possibility of diet as an alternative. The purpose of this study is to analyze the relationship between habitual dietary protein intake and dual task performance in sedentary, recreationally active, and masters athletes. It is hypothesized that the higher protein group will exhibit faster dual task processing speeds in sedentary, recreationally active, and masters athletes older adults.

Methodology

Subjects

Sedentary, recreationally active, and masters athletes adults from the Northwest Arkansas community participated in this study. The University of Arkansas' Institutional Review Board

approved all physical measures and questionnaires. The participants were recruited from various senior centers and clubs in northwest Arkansas. The participants completed an informed consent form and health history questionnaire prior to performing any physical measures.

Measures

Physical Activity Classification. To measure physical activity levels, the Rapid Physical Activity Questionnaire (RAPA) was completed by all participants (Topolski et al., 2006). The RAPA is made up of seven "yes" or "no" questions relating to physical activity and two "yes" or "no" questions regarding resistance training and flexibility. Scores were designated based on validated RAPA classification, with higher scores indicating higher activity levels. A participant was classified as sedentary (SED) when their RAPA score was a 5 or lower. A score of 5 or lower indicates the standard for physical activity by the American College of Sports Medicine (ACSM) has not been met. The standard for sedentary is never or rarely engaging in physical activity or completing less than 30 minutes of exercise a day on three days a week for more than three months (Pescatello et al., 2014). If a participant scores a 6 or 7 on the RAPA but has not competed in a sanctioned event in the last six months, they are physically active by the ACSM standard and are considered recreationally active (RA). Masters athletes are not determined based on their RAPA score; instead, the masters athletes are athletes that have competed in a sanctioned event within the last six months. MAs were currently participating in their individual sports whether it is running, tennis, cycling, or other.

Dietary Classification. The participants were placed into a high or low protein group using the Automated Self-Administered 24-hour dietary recall (ASA-24). The participants provided information on all foods, drinks, and supplements consumed in one 24-hour period. Information on the brand and type of food was documented. Standard serving size, plates, and glasses were used to improve the accuracy of the portion estimate. The Recommended Daily Allowance (RDA) for protein is 0.8-1.2 g/kg body weight. If the participant consumed less than 0.8 g/kg of protein per day, they were placed in the low protein group. If the participant consumed more than 0.8 g/kg of protein per day, they were placed in the high protein group.

Gait Speed. Participants completed four different walking tasks: habitual speed, maximal speed, dual task habitual speed, and dual task maximal speed. Habitual speed is defined as the average, every day walking pace and is the gait a participant would exhibit daily when doing things such as walking to the kitchen. Maximal speed is the fastest speed a participant can safely walk without running. An electronic gate timer was used to document the gait speed. Gait speed was measured over a distance of 10 meters. Using recommendations from previous studies, the participants started 5 meters before the start gate and ended 5 meters after the stop gate so that acceleration and deceleration are not represented in the time (Fritz & Lusardi, 2009). During the dual task trials, participant's gait speed was measured in the same way as the single task trials, but the participant had a second task which was to count backwards by three from a predetermined random number. No number was used twice on the same participant during the trials. For each walking task, two trials were recorded. The average speed of the two trials was calculated in meters per second. An investigator recorded the participant's counting during the dual task trials to determine how many numbers were given and the accuracy of the numbers. Gait speed measurements have reported high inter- and intra-rater reliability (ICC= .90-.96; Steffen et al., 2002).

Statistical Analyses. The independent variable was exercise group (sedentary, recreationally active, masters athlete) and protein group (high, low). The dependent variables were dual task habitual speed and dual task maximal speed. The statistics program used to assess

the data was SPSS version 22 with α <.05. Two multivariate analyses of variance (ANOVA) assessments were used to determine differences between dual task speed (habitual and maximal) between independent variable groups. Follow-up one-way ANOVAs were performed determining differences between exercise groups with regard to dual task performance. Additionally, one-way ANOVAs were used to determine differences in dual task between protein groups. A correlation was calculated to determine if there was a relationship between dual task performance and protein consumed.

Results

A total of 43 participants completed testing. Of the 43 participants, 13 were sedentary, 19 were recreationally active, and 11 were masters athletes. The mean age of all participants (n=43) was 65.16 ± 7.93. Protein consumption per group is reported in Table 1.

Table 1

	N	Protein	Std. deviation
Sedentary	13	.84	.39
Recreationally Active	19	1.14	.44
Masters Athlete	11	1.57	.51
Total	43	1.16	.51

Mean protein consumed by physical activity groups

Note: Protein is reported as g protein/kg body weight per day

MA consumed significantly more protein than SED or RA participants (p < .05). SED and RA did not show differences in amount of protein consumption. The low protein group consumed significantly less g/kg of protein (M=.84, SD=.39) than the high protein group (M=1.30, SD=.50). There was no significant correlation between amount of protein consumed and habitual dual task (r=-.26) or maximal dual task (r=-.21) performance. The high protein group showed nonsignificant benefits for dual task processing speeds over the low protein group in the maximal dual task trial, with the high protein group performing 15% better than the low protein group.

Table 2Dual task performance for high and low protein groups

	Dual Maximal Trial		
	Ν	Mean	Std. Deviation
Low Protein	16	5.71	0.84
High Protein	35	4.90	.81

Note: Mean= m/s to complete dual gait speed trial

Discussion

ASA-24 was chosen because the participants were able to enter the data themselves streamlining the data collection process and potentially increasing accuracy of the results. The program also asks various questions to reduce errors from forgetting to log a food or drink. The program automatically computes the amount of protein consumed; therefore, the researchers are not required to calculate each participant's dietary recall manually. Because one day of dietary reporting may not be the best representation of a person's average intake, the program allows the participant to dictate whether the day reported was much more than usual, usual, or much less than usual. ASA-24 has increased the accuracy of its portion size reporting by including pictures that take the place of 3D food models an interviewer would have if the dietary recall was done in person (Subar et al., 2010). By using a dietary recall, the protein consumption is subject to self-reporting errors; however, the ASA-24 Dietary Recall is deemed a valid measure.

Our sample size was 43; however, when broken down into three physical activity groups, our sample size numbers do not have adequate statistical power. Had there been more subjects, the results of the study could show more significant results; nonetheless, with our current data, there is no significant correlation between protein consumption and dual task performance among different physical activity groups. While the results were not statistically significant, they may have clinical meaning. Perera et al. (2006) showed that for a 10-meter measure of gait speed in older adults, a difference of 0.05 m/s constituted a small meaningful change, and a difference of 0.10 m/s resulted in substantial meaningful difference. When comparing the high and low protein groups for the dual task habitual trial, the high protein group covered the 10-m distance 0.81 seconds faster than the low protein group which was .29 m/s faster. This difference represents a substantial meaningful difference in a clinical setting (Perera et. al 2006). Clinically, the higher protein group may be able to perform activities of daily living more efficiently. Because faster dual task processing is associated with decreased fall risk, clinically, the higher protein group may be at a lower fall risk than the lower protein group, although this was not statistically supported. Physical performance benefits have also been shown as a result of a high protein diet in older adults (Gregario et al., 2013; Mojtahedi et al., 2011).

The MA group (M=1.57) consumed significantly more protein than both the SED (M= 1.14) and RA (M=0.84) group. Tarnopolsky (2008) found that the aging athlete should consume slightly more than the 0.80 g/kg of protein a day that the RDA suggests for all populations in order to optimize muscle strength gains. It is likely that the MA group consistently engages in more strenuous activities related to their sports. Because of consistent strenuous exertion, it makes sense that the MA group would have a higher total caloric intake than the RA or SED groups; furthermore, with the higher caloric intake, it is reasonable that the MA would also

consume more protein each day. Concurrent with this, other studies have suggested that an increase in protein consumption from the RDA recommendation may be beneficial for all aging populations regardless of physical activity level (Lemieux 2013).

References

- Albinet, C. T., Mandrick, K., Bernard, P. L., Perrey, S., & Blain, H. (2014). Improved cerebral oxygenation response and executive performance as a function of cardiorespiratory fitness in older women: A fNIRS study. *Frontiers in Aging Neuroscience Front. Aging Neurosci.*, 6.
- Aragão, F. R., Abrantes, C. G., Gabriel, R. E., Sousa, M. F., Castelo-Branco, C., & Moreira, M. H. (2013). Effects of a 12-month multi-component exercise program on the body composition of postmenopausal women. *Climacteric*, 17(2), 155-163.
- Bhattacharya, T. K., Pence, B. D., Ossyra, J. M., Gibbons, T. E., Perez, S., Mccusker, R. H., ... Rhodes, J. S. (2015). Exercise but not (–)-epigallocatechin-3-gallate or β-alanine enhances physical fitness, brain plasticity, and behavioral performance in mice. *Physiology & Behavior*, 145, 29-37.
- Bhurosy, T., & Jeewon, R. (2013). Food habits, socioeconomic status and body mass index among premenopausal and post-menopausal women in Mauritius. *Journal of Human Nutrition and Dietetics J Hum Nutr Diet*, 26, 114-122.
- Chapman, S. B., Aslan, S., Spence, J. S., Defina, L. F., Keebler, M. W., Didehbani, N., & Lu, H. (2013). Shorter term aerobic exercise improves brain, cognition, and cardiovascular fitness in aging. *Frontiers in Aging Neuroscience Front. Aging Neurosci.*, 5.
- Choquette, S., Dion, T., Brochu, M., & Dionne, I. J. (2012). Soy isoflavones and exercise to improve physical capacity in postmenopausal women. *Climacteric*, *16*(1), 70-77.
- Doi, T., Makizako, H., Shimada, H., Yoshida, D., Ito, K., Kato, T., . . . Suzuki, T. (2011). Brain Atrophy and Trunk Stability During Dual-Task Walking Among Older Adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 67(7), 790-795.
- Fernstrom, J. D., Langham, K. A., Marcelino, L. M., Irvine, Z. L., Fernstrom, M. H., & Kaye, W. H. (2013). The ingestion of different dietary proteins by humans induces large changes in the plasma tryptophan ratio, a predictor of brain tryptophan uptake and serotonin synthesis. *Clinical Nutrition*, 32(6), 1073-1076.
- Filion, M. E., Barbat-Artigas, S., Dupontgand, S., Fex, A., Karelis, A. D., & Aubertin-Leheudre, M. (2012). Relationship between protein intake and dynapenia in postmenopausal women. J Nutr Health Aging The Journal of Nutrition, Health & Aging, 16(7), 616-619.
- Fritz, S., & Lusardi, M. (2009). White paper: "walking speed: The sixth vital sign". *Journal of Geriatric Physical Therapy*, *32*(2), 46–49.
- Froehle, A. W., Hopkins, S. R., Natarajan, L., & Schoeninger, M. J. (2013). Moderate to high levels of exercise are associated with higher resting energy expenditure in communitydwelling postmenopausal women. *Appl. Physiol. Nutr. Metab. Applied Physiology, Nutrition, and Metabolism, 38(11),* 1147-1153.
- Glenn, J. M., Vincenzo, J., Canella, C. K., Binns, A., & Gray, M. (2015). Habitual and Maximal Dual-Task Gait Speeds Among Sedentary, Recreationally Active, and Masters Athlete Late Middle-Aged Adults. JAPA Journal of Aging and Physical Activity, 23(3), 433-437.
- Glenn, J. M., Gray, M., Vincenzo, J. L., Stone, M. (2016). Functional Lower-Body Power: A comparison study between physically inactive, recreationally active, and masters athlete late-middle aged adults. *Journal of Aging and Physical Activity, 24*, 501-507.
- Gregorio, L., Brindisi, J., Kleppinger, A., Sullivan, R., Mangano, K. M., Bihuniak, J. D., . . . Insogn, K. L. (2013). Adequate dietary protein is associated with better physical

performance among post-menopausal women 60–90 years. J Nutr Health Aging The Journal of Nutrition, Health & Aging, 18(2), 155-160.

- Hashimoto, M., Takashima, Y., Uchino, A., Yuzuriha, T., & Yao, H. (2014). Dual Task Walking Reveals Cognitive Dysfunction in Community-dwelling Elderly Subjects: The Sefuri Brain MRI Study. *Journal of Stroke and Cerebrovascular Diseases*, 23(7), 1770-1775.
- Hausdorff, J. M., Schweiger, A., Herman, T., Yogev-Seligmann, G., & Giladi, N. (2008). Dual-Task Decrements in Gait: Contributing Factors Among Healthy Older Adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 63(12), 1335-1343.
- Johnston, R., Poti, J. M., & Popkin, B. M. (2013). Eating and aging: Trends in dietary intake among older Americans from 1977–2010. *J Nutr Health Aging The Journal of Nutrition*, *Health & Aging*, 18(3), 234-242.
- Lemieux, F. C., Filion, M., Barbat-Artigas, S., Karelis, A. D., & Aubertin-Leheudre, M. (2013). Relationship between different protein intake recommendations with muscle mass and muscle strength. *Climacteric*, 17(3), 294-300.
- Mojtahedi, M. C., Thorpe, M. P., Karampinos, D. C., Johnson, C. L., Layman, D. K., Georgiadis, J. G., & Evans, E. M. (2011). The Effects of a Higher Protein Intake During Energy Restriction on Changes in Body Composition and Physical Function in Older Women. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 66A(11), 1218-1225.
- Nagamatsu, L. S., Hsu, C. L., Voss, M. W., Chan, A., Bolandzadeh, N., Handy, T. C., ... Liu-Ambrose, T. (2016). The Neurocognitive Basis for Impaired Dual-Task Performance in Senior Fallers. *Frontiers in Aging Neuroscience*, 8, 20.
- Perera, S., Mody, S. H., Woodman, R. C., & Studenski, S. A. (2006). Meaningful Change and Responsiveness in Common Physical Performance Measures in Older Adults. *Journal of the American Geriatrics Society*,54(5), 743-749.
- Pescatello, L., Arena, R., Riebe, D., & Thompson, P. (2014). ACSM's guidelines for exercise testing and prescription. Baltimore, MD: Wolters Kluwer/Lippincott Williams & Wilkins Health.
- Plummer-D'Amato, P., Kyvelidou, A., Sternad, D., Najafi, B., Villalobos, R. M., & Zurakowski, D. (2012). Training dual-task walking in community-dwelling adults within 1 year of stroke: A protocol for a single-blind randomized controlled trial. *BMC Neurology BMC Neurol*, 12(1).
- Power, A. P., Allen, M. D., Gilmore, K. J., Stashuk, D. W., Doherty T. J., Hepple, R. T., Taivassalo, T., & Rice, C. L. (2016). Motor unit number and transmission stability in octogenarian world class athletes: can age-related deficits be outrun? *Journal of Applied Physiology*, 121(4), 1013-1020.
- Steffen, T.M., Hacker, T.A., & Mollinger, L. (2002). Age- and gender related test performance in community-dwelling elderly people: Six-minute walk test, berg balance scale, timed up & go test, and gait speeds. *Physical Therapy*, 82(2), 128–137.
- Subar, A. F., Crafts, J., Zimmerman, T. P., Wilson, M., Mittl, B., Islam, N. G., ...
 Thompson, F. E. (2010). Assessment of the Accuracy of Portion Size Reports Using Computer-Based Food Photographs Aids in the Development of an Automated Self-Administered 24-Hour Recall. *Journal of the American Dietetic Association*, 110(1), 55-64.

- Tarnopolsky, M. A. (2008). Nutritional Consideration in the Aging Athlete. *Clinical Journal of Sport Medicine*, *18*(6), 531-538.
- Topolski, T.D., LoGerfo, J., Patrick, D.L., Williams, B., Walwick, J., & Patrick, M.M.B. (2006). Peer reviewed: The rapid assessment of physical activity (RAPA) among older adults. *Preventing Chronic Disease*, *3*(*4*), A118–A126.
- Wong, C. N., Chaddock-Heyman, L., Voss, M. W., Burzynska, A. Z., Basak, C., Erickson, K. I., ... Kramer, A. F. (2015). Brain activation during dual-task processing is associated with cardiorespiratory fitness and performance in older adults. *Frontiers in Aging Neuroscience*, 7, 154.
- Yardley, L., Donovan-Hall, M., Francis, K., & Todd, C. (2007). Attitudes and Beliefs that Predict Older People's Intention to Undertake Strength and Balance Training. *The Journals of Gerontology*, 62(2), 119-125.
- Zhu, K., Kerr, D. A., Meng, X., Devine, A., Solah, V., Binns, C. W., & Prince, R. L. (2015). Two-Year Whey Protein Supplementation Did Not Enhance Muscle Mass and Physical Function in Well-Nourished Healthy Older Postmenopausal Women. *Journal of Nutrition*, 145(11), 2520-2526