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Research Article

Nitric Oxide, Gait Speed and Frailty Markers in Functionally Independent Oldest Old

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Abstract

Introduction: The potential mechanisms underlying longevity have become the focus of increasing study. Nitric oxide (NO) triggers multiple signal transduction pathways and helps control numerous cellular functions, influencing frailty, aging and life expectancy. **Objectives:** To evaluate the association of serum nitric oxide levels with gait speed and frailty markers in independent oldest old. **Methods:** Ninety-one community-dwelling oldest old with a mean age of 87.9 years, functional independence and preserved cognition were included. The frailty criteria of Fried et al. were applied. Participants were also classified according morbidity profile into survivors (long-lived with diseases diagnosed at age <80 years) and delayers + escapers (long-lived with diseases diagnosed at age ≥80 years). NO was measured by the chemiluminescence method. **Results:** Participants were predominantly female and classified as survivors. NO levels were higher in robust individuals (mean 234.6 µM) than in pre-frail (215.6 µM) or frail (206.4 µM) subjects. A significant correlation between body mass index (BMI) and NO was detected: for every 1 kg/m^2 increase in BMI, there was an 8.03 uM increase in NO level, controlling for the presence of other predictive variables according to the frailty criteria. **Conclusion:** Increased NO levels were associated with higher BMI values in independent older old and may represent a marker of better health and longevity outcomes.

Keywords: Nitric Oxide; Gait Speed; Oldest Old; Frailty; Body Mass Index

Introduction

Active aging represents a challenge for both society and science. Beyond reaching an advanced age, the goal is to do so without limitations, while maintaining physical and functional independence. Some individuals aged 80 and over, referred to as long-lived or oldest old, are able to cope with diseases and survive potentially catastrophic health outcomes successfully with few sequelae and preserved physical and cognitive functioning. However, multiple factors influence aging that can determine the onset and extent of impairment and negatively impact the aging process.

There are several routes to achieve exceptional longevity. These routes may not imply being free of disease, but are characterized by better coping of the organism with possible pathologies. The results of the study by Thomas Perls et al. involving centenarians from the New England region of the USA [1], showed that more resilient individuals age more independently with less dysfunction. Morbidity profile according to age at diagnosis of certain chronic diseases allowed identification of three distinct groups: long-lived patients with diseases diagnosed at age < 80 years; at 80 -100 years; and at > 100 years.

However, older people may develop the frailty syndrome (aging related) or exhibit the frailty phenotype (secondary to chronic diseases or various etiologists) [2]. These frailty conditions may or may not coexist with disabilities and comorbidities [3-4] and, besides physical impairment, may include cognitive impairment ("cognitive frailty" - cognitive impairment in the absence of a diagnosis of dementia or other underlying neurological disease) [2].

Frailty in the elderly population seems, in part, to be related to the presence of chronic inflammatory status [5]. This inflammatory activity leads to slow progressive tissue injury and appears to be closely associated with the risk of all-cause mortality in this group [6]. Many studies hold that this chronic inflammation represents one of the biological mechanisms responsible for the decline in physical function in this population [7-9].

Addressing the need to identify risk populations as early as possible, Studenski et al. determined the degree to which gait speed (GS) of an older person with complete or partial functional independence, explains variability in survival, after adjusting for sex and age [10]. Several biochemical and inflammatory markers have also been studied as markers of health outcome, such as serum levels of ultrasensitive C-reactive protein (CRP) [11], a preclinical marker of cardiovascular system diseases (CAD).

Some studies have shown reduced serum nitric oxide (NO) levels in older adults and individuals with CAD and associated risk factors, such as hypercholesterolemia, hyperhomocysteinemia, arterial hypertension (AH) and diabetes mellitus (DM) [12-14]. In a review published in the Journal of the American Geriatrics Society, the authors reinforce the concept of "vascular age", contesting the established view that an individual's chronological age is an independent risk factor for CAD [15]. In the study, impaired NO bioavailability is considered a predisposing factor for endothelial lesions, associated with AH and hypercholesterolemia. Based on this premise, individuals able to synthesize NO effectively, naturally or through the use of specific medication, have a lower risk of developing CAD and having greater survival.

Recent studies have begun to address the role of vascular endothelium integrity and its correlation with NO synthesis and increased life expectancy [16], in general and also through the expression of specific genes related to longevity [17-19].

However, no studies to date have clarified the relationship of NO and vascular endothelium with frailty syndrome or gait speed. This dearth of studies prompted the present investigation exploring the relationship among these variables in independent oldest old, analysing factors associated with body mass index (BMI) and morbidity profiles in this population.

Method

Independent community-dwelling older adults aged 80 or over were recruited through the media (newspapers, magazines, radio and television) and personal contact to take part in the follow-up program at the UNIFESP Geriatric and Gerontology Discipline in 2017. The inclusion criteria were: independent for walking; no acute or chronic decompensated diseases, neoplasms, dementia or chemotherapy, radiotherapy or dialysis treatment. The study was approved by the Research Ethics Committee of UNIFESP and all participants signed an informed consent form. Subjects unable to answer questionnaires and assessments were not included.

All participants underwent serum assay collection and both physical and functioning assessments. For assay collection, all participants adhered to a 12-hour fasting period and abstained from using alcohol in the preceding 48 hours. Serum 25 hydroxy-vitamin D (25 (OH) VitD)) level was determined by radioimmunoassay (DiaSorin Inc., Stillwater, USA) and usCRP by nephelometry.

NO was determined by the chemiluminescence technique, a highly sensitive method for detecting this molecule; deproteinized serum samples were used for reading on the device (Nitric Oxide Analyzer - NOATM 280, Sievers Instruments, Inc. Boulder, CO, USA) at the research laboratory of the Nephrology Discipline of the UNIFESP.

The criteria for physical frailty were assessed using the Fried protocol (20):

- **Weight Loss:** weight assessment in the previous year and in the consultation in question to investigate whether there was a loss of 5% or more of the individual's total weight unintentionally.
- **Self-reported exhaustion:** based on responses to 2 questions from the Center for Epidemiological Studies (CESD) depression scale validated for older Brazilian adults (21).
- **Physical activity:** level of physical activity was assessed by the Minnesota Leisure Time Physical Activities Questionnaire (MLTPA-Q) translated and adapted for use in the Brazilian older population (22).
- **Gait Speed (GS):** participants were asked to cover a timed distance of 4 meters in their usual stride. The same procedure was repeated twice and the best performance was taken to be a positive frailty criterion if the individual had a walking speed below 0.8 m/s (10).
- **Hand Grip strength (HG):** a hydraulic dynamometer was used following the application protocol of the American Society of Hand Therapy. Three measurements were taken with an interval of one minute between each and the average of the three measurements was used. Cut-off points for frailty were stratified according to gender and the individual´s body mass index (BMI) (20).

The oldest old were classified according to morbidity profile (1) see in (Chart 1) into:

- **survivors:** long-lived with diseases diagnosed at age < 80
- **delayers + escapers:** long-lived with diseases diagnosed at age ≥ 80r

Profile	Pathologies assessed for age at onset
Profile 1* * Most common causes of mortality in older persons	Heart disease, stroke and neoplasms (exceptskin cancer)
Profile 2** ** Includes most common causes of mortality plus chronic diseases	Heart disease, stroke, neoplasms, high blood pressure, Parkinson's disease, chronic obstructive pulmonary disease, diabetes, thyroid disorders and osteoporosis

Chart 1: Morbidity profiles

Statistical Analysis

Comparisons of means between groups were performed using Student's t-test (for comparison of two means) or analysis of variances - ANOVA (for comparison of more than two means). Both Student's t-test and ANOVA are based on the assumption of normality in data distribution and homoscedasticity (equality of variance between groups), verified using the Kolmogorov-Smirnov test and Levene's test, respectively. In the event of violation of the homoscedasticity assumption, the degrees of freedom of the F statistic were corrected using the Brown-Forsythe correction test [23]. In the event of violation of the normality assumption on Student's t-test, the Mann-Whitney non-parametric test was used as an alternative. The existence of associations between two categorical variables was verified using the chi-square test. The linear association between two numerical variables was assessed using Pearson's correlation.

Multiple linear regression was used to assess the effects of frailty components (feeling of exhaustion or tiredness, loss of more than 5% in weight), MLTPA-Q, HG and GS or frailty classification adjusted for age, sex, BMI, AH, DM, dyslipidemia (DLP), usCRP, 25 (OH) VitD, vitamin D replacement and morbidity profiles 1 and 2 (explanatory variables) on NO (dependent variable). Assumptions of normality in the distribution were confirmed using the Kolmogorov-Smirnov test.

Winsorization of variables was carried out when indicated. Winsorization is a procedure devised by the biostatistician C. P. Winsor which entails trimming the extreme values (above or below the defined minimum and maximum percentiles), substituting these for the smallest and largest values remaining in the distribution. In this study, the 97.5 percentile was considered and the corresponding value of which was 557.8 μM.

For all statistical tests, a significance level of 5% was adopted.

Statistical analyses were performed using the statistical software SPSS 20.0.

Results

The oldest old were predominantly female and classified as survivors with AH, DLP and DM proving the most prevalent comorbidities (Table 1). None of the participants had weight loss > 5%, all were active according to the Minnesota scale, and had good hand grip strength. However, participants underperformed for GS and 78% were pre-frail or frail. Although not reaching statistical significance, differences in mean NO according to sex and frailty classification were evident.

Two models of multiple linear regression were fitted. The first of these models incorporated the predictor variables age, sex, BMI, comorbidity (AH, DM and DLP), CRP, 25OHVITD, vitamin D replacement, morbidity profile classifications 1 and 2, and the five components of frailty (Table 2). The second model was similar to the first, except the five frailty components were replaced by global frailty classification (Table 2). For every 1 kg/m2 increase in BMI, there were 8.39 (uM) and 8.03 (uM) increases in NO on the models, respectively, controlling for the presence of the other predictive variables.

The distribution of the oldest old for NO terciles, according to age group and morbidity profiles 1 and 2, revealed no associations (Table 3).

	N	%	Mean	Standard Deviation	Minimum	Maximum	1st. Quartile	Median	3rd. Quartile	p
Age (years)	91		87.9	4	81	103	85	87	90	
Minnesota Score	91		675.7	884.5	0	4457.5	0	411.6	982	
BMI (kg/m2)	91		26.06	4.26	18.58	39.06	23.24	25.7	28.18	
usCRP (mg/dL)	83		2.92	4.2	0.04	28.3	0.69	1.64	2.8	
25OHVITD (ng/mL)	69		35.18	13.21	9.1	78	24.7	35.8	43.6	
NO (uM)	91		217.43	115.16	45.63	557.75	130.8	193.85	270.1	
Sex/NO level (uM)										0.073
Male	28	30.8	185	94.4	45.6	443	115.9	160.6	239.7	
Female	63	69.2	231.9	121.2	81	557.8	140	204.6	284	
Age Group/NO level (uM)										0.33
81 - 87 years	48	52.7	228.6	127.8	85	557.8	130.4	201	297.3	
≥ 88 years	43	47.3	204.9	99.2	45.6	480.1	130.8	187	247	
AH /NO level (uM)										0.496
Yes	67	73.6	222.4	123.6	45.6	557.8	129.5	193.9	270.1	
No	24	26.4	203.6	88.2	88	443	138.5	198.3	277.3	
DM /NO level (uM)										0.968
Yes	26	28.6	218.2	101.4	81	555.5	152.3	207	273.6	
No	65	71.4	217.1	120.9	45.6	557.8	128.8	187	272.7	
DLP/NO level (uM)										0.385
Yes	45	49.5	228.1	132	45.6	557.8	133.8	199	277.3	
No	46	50.5	207	96.2	81	523.4	126.2	192.9	268.4	
Vitamin D Replacement/NO level (uM)										0.424
Yes	73	80.2	222.3	115.6	81	557.8	135.5	193.9	287.5	
No	18	19.8	197.9	114.5	45.6	557.8	125.3	178.8	246.4	
Morbidity Profile1/ NO level (uM)										0.89
Survivor	16	17.6	213.8	125.5	85	557.8	129.1	182.2	235.9	
Delayer/Escaper	75	82.4	218.2	113.7	45.6	557.8	133	197	284	
Morbidity Profile 2 /NO level (uM)										0.999
Survivor	51	56	217.4	107.3	85	557.8	134.7	193.9	255	
Delayer/Escaper	40	44	217.5	125.8	45.6	555.5	126.1	187.2	289	
Frailty - component/NO level (uM)										
Weight loss > 5%										0.989
Yes	12	13.2	217.9	111.6	88	443	134.9	193	267.9	
No	79	86.8	217.4	116.4	45.6	557.8	130.8	193.9	270.1	
Exhaustion										0.932
Yes	23	25.3	219.2	121.2	45.6	523.4	126	203.1	284	
No	68	74.7	216.8	114	81	557.8	138.5	187	268.4	
Minnesota										0.488a
Active	50	54.9	221.9	112.7	88	557.8	132.5	204.8	286	
Inactive	41	45.1	212	119.3	45.6	555.5	127.9	175.5	259.1	
GS										0.68
Adequate	41	45.1	223.1	133.3	85	557.8	125.8	187	301	
Inadequate	50	54.9	212.8	99	45.6	555.5	136.8	200.3	257.1	
HG										0.431
Adequate	57	62.6	224.8	125.1	45.6	557.8	131.8	204.6	287.5	
Inadequate	34	37.4	205	96.7	81	523.4	130.5	182.2	246.4	
Frailty – classification										0.720b
Frail	23	25.3	206.4	102.2	45.6	443	126.3	192	284	
Pre-frail	48	52.7	215.6	110.9	85	555.5	138.8	190.4	254.5	
Robust	20	22	234.6	140.9	96	557.8	128.6	204.8	302.8	

BMI= body mass index, usCRP= ultra-sensitive C-reactive protein, 25OHVITD= 25 hydroxy vitamin D, AH= arterial hypertension, DM= diabetes mellitus, DLP= dyslipidemia, GS= gait speed, HG= Hand Grip strength, NO= nitric oxide; 1= comorbidities diagnosed before age 80; 2= comorbidities diagnosed after age 80. *Survivor*= long lived with diseases diagnosed at <80 years; *Delayer/Escaper*= long lived with diseases diagnosed at ≥80 years. p – descriptive level of Student´s *t*-test, Mann-Whitney ([a]) or ANOVA ([b]). AH (arterial hypertension), DM (diabetes mellitus), DLP(dyslipidemia), GS (gait speed), HG (Hand Grip strength), NO (nitric oxide) Winsorization applied for NO. BMI, usCRP, 25OHVITD

Table 1: Distribution of oldest old by demographic and clinical characteristics and classification of morbidity profile and serum NO levels (uM), expressed as percentages and summary measures.

	Initial Model - frailty components	
	Coefficient (95%CI)	**p**
Constant	203.29 (122.61 ; 283.97)	<0.001
Gender male (ref.= female)	-47.97 (-115.79 ; 19.85)	0.162
Age (years)	3.81 (-4.91 ; 12.53)	0.384
BMI (kg/m^2)	**8.39 (1.33 ; 15.44)**	**0.021**
CRP	0.90 (-5.36 ; 7.16)	0.774
25OHVITD	0.25 (-2.03 ; 2.52)	0.830
AH (ref.=no)	31.07 (-39.45 ; 101.59)	0.380
DM (ref.=no)	-23.06 (-89.11 ; 42.99)	0.486
DLP (ref.=no)	24.41 (-30.98 ; 79.80)	0.380
No Vitamin D replacement (ref.=yes)	8.49 (-70.53 ; 87.51)	0.830
***Survivor*–morbidity profile 1 (ref. = *Delayer/Escaper*)**	23.70 (-57.83 ; 105.23)	0.562
***Survivor* – morbidity profile 2 (ref. = *Delayer/Escaper*)**	8.79 (-56.88 ; 74.45)	0.789
Frailty components		
Weight Loss > 5% (ref.=no)	11.51 (-85.91 ; 108.92)	0.813
Exhaustion (ref.= no)	-13.33 (-79.16 ; 52.51)	0.686
Minnesota (ref.= active)	-31.77 (-93.67 ; 30.13)	0.308
GS inadequate (ref.=adequate)	16.52 (-49.02 ; 82.06)	0.615
HG inadequate (ref.=adequate)	-45.95 (-109.78 ; 17.88)	0.154
	Model - frailty classification	
	Coefficient (95%CI)	**p**
Constant	183.62 (109.27 ; 257.96)	<0.001
Gender male (ref.= female)	-54.23 (-119.49 ; 11.03)	0.101
Age (years)	4.31 (-3.81 ; 12.43)	0.292
BMI (kg/m^2)	**8.03 (1.19 ; 14.88)**	**0.022**
CRP	0.76 (-5.38 ; 6.91)	0.805
25OHVITD	0.26 (-1.97 ; 2.49)	0.816
AH (ref.=no)	26.20 (-40.96 ; 93.35)	0.437
DM (ref.=no)	-18.07 (-82.49 ; 46.35)	0.576
DLP (ref.=no)	24.48 (-29.79 ; 78.75)	0.370
No Vitamin D replacement (ref.=yes)	0.87 (-74.71 ; 76.46)	0.982
***Survivor* – morbidity profile 1 (ref. = *Delayer/Escaper*)**	21.20 (-58.81 ; 101.2)	0.597
***Survivor* – morbidity profile 2 (ref. = *Delayer/Escaper*)**	6.53 (-56.81 ; 69.88)	0.837
Frailty (ref.= pre-frail		0.512
Frail	-18.81 (-85.57 ; 47.96)	0.574
Robust	27.79 (-46.33 ; 101.91)	0.455

N=67.

BMI= body mass index, usCRP= ultra-sensitive C-reactive protein, 25OHVITD= 25 hydroxy vitamin D, AH= arterial hypertension, DM= diabetes mellitus, DLP= dyslipidemia, GS= gait speed, HG= Hand Grip strength, NO= nitric oxide; 1= comorbidities diagnosed before age 80; 2= comorbidities diagnosed after age 80. Survivor= long lived with diseases diagnosed at < 80 years; Delayer/Escaper= long lived with diseases diagnosed at ≥ 80 years

Table 2: Estimates of regression model for NO – frailty components and classification

	NO terciles						p
	1st tercile(≤ 145)		2nd tercile(145 -- 238)		3rd. Tercile(>238)		
	N	%	N	%	N	%	
Age group	**31**	**34.1**	**30**	**33.0**	**30**	**33.0**	0.710
81 - 87 years	17	35.4	14	29.2	17	35.4	
≥ 88 years	14	32.6	16	37.2	13	30.2	
Morbidity Profile 1	**31**	**34.1**	**30**	**33.0**	**30**	**33.0**	0.755
Survivor	6	37.5	6	37.5	4	25.0	
Delayer/Escaper	25	33.3	24	32.0	26	34.7	
Morbidity Profile 2	**31**	**34.1**	**30**	**33.0**	**30**	**33.0**	0.802
Survivor	16	31.4	18	35.3	17	33.3	
Delayer/Escaper	15	37.5	12	30.0	13	32.5	

NO= nitric oxide, 1= comorbidities diagnosed < 80 years; 2= comorbidities diagnosed ≥ 80 years. Survivor= long lived with diseases diagnosed at < 80 years; Delayer/Escaper= long lived with diseases diagnosed at ≥ 80 years

Table 3: Distribution of oldest old by NO tercile, according to age group andmorbidity profiles 1 and 2.

Discussion

We observed a positive correlation between increase in NO and BMI (p = 0.02). There may be a relationship between better outcomes and integrity of vascular endothelium, represented by higher levels of NO. In a 2017 meta-analysis, Winter et al. observed an increase in mortality, starting at a BMI of 23 kg / m2 and reaching significance at 28 kg / m2, in individuals aged < 65 years. By contrast, in older adults there was a reduced risk of mortality at a BMI of between 23 kg / m2 and 29 kg / m2, with a clear increase in mortality for BMI < 22 kg / m2 [24]. One possible hypothesis is that late-life energy reserves can be protective [25].

Several studies have suggested serum NO levels are positively correlated with BMI in overweight individuals [27-29]. It has also been demonstrated that the precursor enzymes for NO synthesis (NO endothelial synthase-eNOS and inducible-iNOS) are present in subcutaneous adipose tissue [30] and that their inhibition can induce lipolysis of this tissue [31]. This factor might explain the elevated NO production seen in the long- lived population studied without, however, representing a marker of direct endothelial injury.

The CRP levels found indicated long-lived individuals with low inflammatory pattern and good endothelial integrity, but the prevalence of frailty and pre-frailty was high and the majority of subjects had low GS, suggesting a higher risk of mortality. However, some of these individuals may fall into the category of slow walkers, exhibiting slow gait yet good survival, even with GS < 0.8 m / s [10].

The potential mechanisms underlying longevity have become a focus of increasing study. There are several reasons that might explain exceptional longevity. Good nutritional status associated with physical activity is clearly a major health promoter. Cellular and endothelial senescence seem to be closely linked to the production of certain proteins related to longevity, such as eNOS. One of the molecules that regulates the synthesis of eNOS is sirtuin-1 (Sirt-1), a recognized longevity marker [17]. There seems to be a correlation of decreased Sirt-1 and consequent decrease in eNOS synthesis with age-related endothelial dysfunction [18]. Sirtuins (SIRTs) have been shown to regulate cell senescence and are generally considered to be longevity factors, based on experimental observation that increased expression of orthologs in the gene called the Silent Information Regulator 2 (Sir2) is sufficient to increase life span in lower organisms [17,19].

The average level of circulating NO was higher in women and in the oldest old classified as robust. In a 2013 study investigating the relationship between bioavailability of serum nitrite in older women after a 400m walk, the researchers observed a decrease in the levels of NO metabolites. Twenty percent of the older women, however, showed an increase in serum nitrite levels [32]. Considering the concept of vascular age [33], satisfactory levels of

NO could be indicators of good health status, perhaps explaining the feminization of aging. According to this concept, older women seem better able to cope with age-related diseases than men, who seem more likely to die from potentially lethal diseases before age 80 [24].

This population is difficult to recruit and no previous Brazilian studies have evaluated older people aged over 80 years who are independent, have compensated chronic illnesses and no serious illnesses or dementia. Unlike previous studies, the present investigation explored a large number of variables that can influence serum NO levels, and the methodology used to measure serum NO levels was more sensitive than techniques employed in other studies, where the sensitivity of the Nitric Oxide Analyzer (NOA) used for measuring NO in the gas phase is approximately 1 picomol.

Our findings are highly relevant and may be the result of good health status and potential for longevity.

Conclusion

Higher levels of NO were associated with higher BMI values in independent older old and may represent a marker of better health and longevity outcomes. These findings should be confirmed in longitudinal studies.

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References

1. Evert J, Lawler E, Bogan H, Perls T (2003) Morbidity Profiles of Centenarians: Survivors, Delayers, and Escapers. J Gerontol A Biol Sci Med Sci 58: 232-7.
2. Luigi Ferruci; Elisa Fabbri; Jeremy D Waltson. Frailty. In: Halter Jeffrey, Stephanie Studenski JO, editor. Hazzard´s Geriatric Medicine and Gerontology. 7th Editio. McGraw-Hill Education; 2017. p. 691–708.
3. LP F (1992) Conference on the physiologic basis of frailty. April 28, 1992, Baltimore, Maryland, U.S.A. Introduction. Aging (Milano) 4: 251-2.
4. Walston J, Hadley EC, Ferrucci L, Guralnik JM, Newman AB, et al. (2006) Research agenda for frailty in older adults: Toward a better understanding of physiology and etiology: Summary from the American Geriatrics Society/National Institute on Aging research conference on frailty in older adults. J Am Geriatr Soc 54: 991-1001.
5. Franceschi C, Bonafè M, Valensin S (2000) Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the filling of immunological space. Vaccine 18: 1717-20.
6. Bruunsgaard H, Skinhéj P, Pedersen AN, Schroll M, Pedersen BK (2000) Ageing, tumour necrosis factor-alpha (TNF- a) and atherosclerosis. Clin Exp Immunol 121: 255-60.
7. Roubenoff R (2003) Catabolism of aging: is it an inflammatory process? Curr Opin Clin Nutr Metab Care 6: 295-9.
8. Boren E, Gershwin ME (2004) Inflamm-aging: autoimmunity, and the immune-risk phenotype. Autoimmun Rev 3: 401-6.
9. Cappola AR, Xue QL, Ferrucci L, Guralnik JM, Volpato S, et al. (2003) Insulin-like growth factor I and interleukin-6 contribute synergistically to disability and mortality in older women. J Clin Endocrinol Metab 88: 2019-25.
10. Studenski S, Perera S, Patel K, Rosano C, Faulkner K, et al. (2011) Gait Speed and Survival in Older Adults. JAMA 305: 50-8.
11. Ridker PM (2003) Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 107: 363-9.
12. Keaney JF, Vita JA (1995) Atherosclerosis, oxidative stress, and antioxidant protection in endothelium-derived relaxing factor action. Prog Cardiovasc Dis 38: 129-54.
13. Lyons D (1997) Impairment and restoration of nitric oxide-dependent vasodilation in cardiovascular disease. int J Cardiol 62 Suppl 2: S101-9.
14. Taddei S, Virdis A, Ghiadoni L, Salvetti A (1998) Endothelial dysfunction in hypertension: fact or fancy? J Cardiovasc Pharmacol 32 Suppl 3: S41-7.
15. Walsh T, Donnelly T, Lyons D (2009) Impaired endothelial nitric oxide bioavailability: A common link between aging, hypertension, and atherogenesis? J Am Geriatr Soc 57: 140-5.
16. Puca AA, Spinelli C, Accardi G, Villa F, Caruso C (2018) Centenarians as a model to discover genetic and epigenetic signatures of healthy ageing. Mech Ageing Dev 174: 95-102.
17. Huxley A. Sirtuins and Longevity. 2012;2(1):1–4.
18. Donato AJ, Magerko KA, Lawson BR, Durrant JR, Lesniewski LA, et al. (2011) SIRT-1 and vascular endothelial dysfunction with ageing in mice and humans. J Physiol 18: 4545-54.
19. Potente M, Dimmeler S (2008) NO targets SIRT1: A novel signaling network in endothelial senescence. Arterioscler Thromb Vasc Biol 28: 1577-9.
20. Fried PL, Tangen MC, Walston J, Newman BA, Hirsch C, et al. (2001) Frailty in Older Adults: Evidence for a Phenotype. J Gerontol A Biol Sci Med Sci 56: 146-56.
21. Batistoni SST, Neri AL, Cupertino APFB (2007) Validade da escala de depressão do Center for Epidemiological Studies entre idosos brasileiros Validity of the Center for Epidemiological Studies Depression. Rev Saúde Pública 41: 598-605.
22. Lustosa LP, Pereira DS, Dias RC, Britto R, Parentoni a, et al. (2011) Tradução e adaptação transcultural do Minnesota Leisure Time Activities Questionnaire em idosos. Geriatr Gerontol 5: 57-65.
23. Brown MB, Forsythe AB (1974) Robust tests for equality of variances. J Am Stat Assoc 69: 364-7.
24. Wingard DL (1984) The sex differential in morbidity, mortality, and lifestyle. Annu Rev Public Heal 5: 433-58.
25. Batsis JA, Mackenzie TA, Emeny RT, Lopez-Jimenez F, Bartels SJ (2017) Low Lean Mass With and Without Obesity, and Mortality: Results From the 1999–2004 National Health and Nutrition Examination Survey. J Gerontol A Biol Sci Med Sci 72: 1445-51.
26. Landi F, Zuccalà G, Gambassi G, Incalzi RA, Manigrasso L, et al. (1999) Body mass index and mortality among older people living in the community. J Am Geriatr Soc 47: 1072-6.

27. David CN, Mello RB, Bruscato NM, Moriguchi EH (2017) Overweight and Abdominal Obesity Association With All-Cause and Cardiovascular Mortality in the Elderly Aged 80 and Over: a Cohort Study. J Nutr Health Aging 21: 597-603.

28. Foroumandi E, Alizadeh M, Kheirouri S, Jafarabadi MA (2019) Exploring the role of body mass index in relationship of serum nitric oxide and advanced glycation end products in apparently healthy subjects. PLoS One 14: e0213307.

29. Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Janowska J, Zurakowski A (2004) Serum concentrations of nitric oxide, tumor necrosis factor (TNF)-α and TNF soluble receptors in women with overweight and obesity. Metabolism 53: 1268-73.

30. Elizalde M, Rydén M, Van Harmelen V, Eneroth P, Gyllenhammar H, et al. (2000) Expression of nitric oxide synthases in subcutaneous adipose tissue of nonobese and obese humans. J Lipid Res 41: 1244-51.

31. Andersson K, Gaudiot N, Ribiere C, Elizalde M, Giudicelli Y, et al. (1999) A nitric oxide-mediated mechanism regulates lipolysis in human adipose tissue in vivo. Br J Pharmacol 126: 1639-45.

32. Dobrosielski DA, Presley T, Perlegas A, Marsh AP, Kim-Shapiro D, et al. (2010) Plasma nitrite response in older women to a physical function test. Aging Clin Exp Res 22: 383-6.

33. Dohi Y, Thiel MA, Bühler FR, Luscher Tf (1990) Activation of endothelial L-arginine pathway in resistance arteries: effect of age and hypertension. Hypertension 16: 170-9.