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Markers of inflammation, Vitamin E and peripheral nervous system function:

The InCHIANTI study

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Abstract

Background—Aging of the peripheral nervous system is associated with several morphologic and functional changes, including a decrease of the nerve conduction velocity. There is evidence that these changes contribute to age-related-decline in muscle strength, sensory discrimination, and autonomic responses. The aim of this study was to characterize the decline in nerve conduction velocity in the peripheral nervous system over the aging process and to identify factors that, independent of age, affect nerve conduction velocity.

Methods—We measured motor nerve conduction velocity of the right superficial peroneal nerve using a standard neurophysiologic technique in a population-based sample of subjects aged between 20 and 103 years old enrolled in the InCHIANTI study.

Results—Average conduction velocities in the peripheral nerve decreased linearly with age in both sexes. We found that diabetes, cognitive impairment, uric acid, sIL-6R and α-tocopherol were significant predictors of nerve conduction velocity independently of the potential confounding effect of age, sex, sex × age interaction term, height, lymphocytes, neutrophils number, α1 and α2-globulin serum protein.

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Conclusion—Our findings are consistent with the hypothesis that inflammation and inadequate antioxidant defenses are associated with accelerated decline of nerve conduction velocity over the aging process.

Keywords

Inflammation; Vitamin E; Peripheral nervous system; Aging

1. Introduction

Aging is characterized by a decline in function of multiple physiological systems and progressive exhaustion of their functional reserve. The causes of this decline are still unclear but it has been proposed that increased oxidative stress, disturbances in energy metabolism, and a primary dysregulation of the immune system might play an important role [22].

A number of observational studies have shown an age-associated decline in peripheral nervous system (PNS) function. Longitudinal assessment performed in subjects of different ages demonstrated that nerve conduction velocity (NCV) and signal amplitude decrease with age even in subjects free of diabetes and other major diseases [24]. Such decline has been attributed to structural changes, such as loss of myelinated and unmyelinated fibers and decreased production of the major myelin proteins with subsequent myelin deterioration [33].

Factors that have been associated with reduced NCV include diabetes, inflammatory disease, smoking, alcohol abuse and chronic infection [1,23]. However, no previous study tested the hypothesis that independently of age, a proinflammatory and pro-oxidative state, characterized by circulating levels of inflammatory markers and low circulating levels of antioxidants, is associated with reduced NCV.

The aim of this study was to evaluate whether the decline in NCV occurs linearly over the entire life span and to verify whether, independent of age and chronic diseases, high levels of inflammatory markers and low levels of Vitamin E, the most important lipophilic antioxidant, are associated with lower NCV.

2. Population and methods

InCHIANTI is an epidemiological study of factors contributing to the decline of mobility in late life. The InCHIANTI study population is a representative sample of the population living in Greve in Chianti and Bagno a Ripoli, two small towns located in the Chianti countryside of Tuscany, Italy. The participants were all European of *Caucasian* race. The study design and data collection have been previously described elsewhere [6]. Briefly, 1270 persons aged 65 years or more were randomly selected from the population registry of the two sites. Another 29 subjects were selected randomly from among those who were aged 90 years or older. Finally, men and women sampled randomly from the age strata 20–29, 30–39, 40–49, 50–59 and 60–64 years were sequentially invited to participate in the study until at least 30 men and 30 women for each decade from 20 to 59, and 10 men and 10 women aged 60 to 64 had been enrolled (Table 1).

Of the 1530 subjects originally sampled, 1453 (94%) agreed to participate in the study. Of these, 1263 underwent standard surface electroneurography of the right peroneal nerve. Due to missing data 57 subject were excluded from the subsequent analyses.

The final study population included 1206 persons, 543 men and 663 women, dispersed over a wide age-range (21–96 years). The study protocol was examined and approved by the INRCA

ethical committee. All participants received a detailed description of the purpose and design of the study and all signed informed participation consent.

2.1. Assessment

The study protocol included: a home interview, a validated food frequency questionnaire [26], a clinical test session, a medical examination and a blood sample collection after at least 8 h of fasting. Both cytokines and Vitamin E were measured from frozen (−80 °C) specimens.

2.2. Electromyography

The measure of nerve motor conduction velocity was performed on the right superficial peroneal nerve using standard neurophysiologic equipment (EMG Myto, EBNeuro, ESAOTE Florence, Italy). The temperature of testing room was kept at 26–27 °C so that the testing leg is maintained at physiologic temperature. The measurements were obtained while dorsal foot skin temperature was between 30 and 34 °C [13,27]. If necessary the skin over the muscle was warmed up with an infrared lamp.

A disposable surface recording electrode was placed on the dorsum of the foot, over the belly of the *extensor digitorum brevis* (in the anterior lateral aspect of the proximal midtarsal area); the peroneal nerve was stimulated with a bipolar electrode, distal stimulation was applied about 8 cm proximal to the pickup, just lateral to the tibialis anterior tendon. More proximally the nerve was stimulated just below the head of the fibula as the nerve curves around the bone. A ground electrode was positioned in the between the stimulating and the recording electrodes [3,10] (Fig. 1).

In both cases, the stimulation started with a very mild electrical impulse, to check whether the position of the electrodes was appropriate. Then, the stimulus was progressively increased, until the registered muscular response (a sinusoid) reached maximum amplitude.

The following parameters of nerve conduction studies were measured: (a) the amplitude of the compound muscle action potential (CMAP), which is related to the number of axons that conduct impulses from the stimulus point to the muscle and the number of functioning motor endplates [27]. It was measured from peak to peak of the action potential [19,27]; (b) the nerve conduction velocity (NCV), which is calculated by dividing the length of the nerve segment between the two stimulation points by the difference between the proximal and distal time latency, which reflects the conduction velocity of the fastest motor axons [13,27].

During test measurement electrical averaging was performed regularly to reduce the signal to noise ratio [13].

2.3. Electromyograph setting

According to literature electromyograph setting were: frequency 8 Hz to 8 kHz; sweep speed 5 ms/div; gain 1.000 μV [13].

2.4. Cytokines measurement

Chronic inflammation is associated with a broad spectrum of degenerative age related diseases such as Alzheimer disease, congestive heart failure, atherosclerosis, and myocardial infarction; it has been observed that aging “per se” is associated with the development of a pro-inflammatory state [2,7,8,17].

A number of cytokines were evaluated on the whole InCHIANTI sample to obtain information both the pro and anti-inflammatory response.

The serum interleukin 6 (IL-6), Tumor Necrosis Factor-α (TNF-α), interleukin 1β (IL-1β) levels were measured by enzyme linked immunosorbent assay (ELISA) using ultrasensitive commercial kits (Human Ultrasensitive, BIOSOURCE International Inc.). The detectable limits were: 0.10 pg/ml for IL-6, 0.09 pg/ml for TNF-α and 0.01 pg/ml for IL-1β.

The serum interleukin 6 receptor (sIL-6R) level was determined by ELISA kit (Cytoscreen™, Human sIL-6R BIOSOURCE International Inc.) and the minimum detectable level was 8.00 pg/ml.

The serum interleukin 1 receptor antagonist (IL-1ra) level was detected by ELISA method using commercial kits (EASIA™ ELISA Human IL-1ra, BIOSOURCE International Inc.). The minimum detectable concentration was 4.00 pg/ml. The serum interleukin 10 (IL-10) was detected by Human IL10 CytoSETS™ ELISA kits (BIOSOURCE International Inc.). The minimum detectable concentration was 1.00 pg/ml.

2.5. α-Tocopherol measurement

Vitamin E (α-tocopherol) plasma concentration was measured by reverse-phase high performance liquid chromatography (HPLC) as previously described [16]. Briefly, 100 μl of plasma were mixed with 100 μl ethanol; after vortexing, tocopherol was extracted into 500 μl hexane containing 0.002% butylated hydroxyl toluene (BHT) (Sigma St. Louis, MO). Tocol (a gift from Hoffman La Roche, Nutley, NJ), was added to the mixture as an internal standard. Samples were centrifuged at 800 rpm for 5 min at 4 °C. The supernatant was collected and dried under a stream of nitrogen gas, and reconstituted in 100 μl of methanol. Tocopherols were separated by high performance liquid chromatography (HPLC) using a 3 μm C18 reverse phase column (Perkin-Elmer, Norwalk, CT). The mobile phase, delivered at a flow rate of 1.0 ml/min, consisted of 1% water in methanol, containing 10 mmol/l lithium perclorate. Samples were injected with an autosampler, 1100 series, Hewlett Packard. Eluted peaks were detected at an applied potential of +0.6 V by a LC 4B amperometric electrochemical detector (Bioanalytical System, West Lafayette, IN). Peaks were integrated with a ChemStation software (Hewlett Packard). Tocopherol (α-tocopherol) concentration was expressed in μmol/l. Reproducibility and accuracy of the assay was tested by analyzing representative samples in triplicate from a sample provided by the American Association for Laboratory Accreditation, Washington, DC, USA containing known concentration of α-tocopherol. Intra-and inter-batch coefficients of variation were 3% and 4.2%, respectively.

2.6. Covariates

Average daily alcohol consumption was evaluated by means of the food frequency questionnaire. Smoking was categorized into: never smoker, past smoker and smoker. Based on self report, the diagnosis of several chronic medical conditions was established using pre-defined criteria that combined information from medical history, physical exam and medical records. The list of diseases includes: congestive heart failure (CHF), angina, myocardial infarction (MI), hypertension, stroke, diabetes, deep venous thrombosis, parkinson disease, peripheral artery diseases and hip fracture [25].

Cognitive function was assessed using the Mini Mental Status Examination (MMSE) corrected for age and education. Participants who scored 19 or less were considered as “cognitive impaired”.

Height was clustered according to sex specific tertiles of distribution; ranges for male were 163 and 169 cm, ranges for female were 150 and 158 cm.

The results of blood tests were categorized according to tertiles of distribution: (a) α1 globulin (% total protein) (<2.4, 2.4–2.7, >2.7); (b) α2 globulin (% total protein) (<10.5, 10.5–11.6,

>11.6); (c) albumin (% total protein) (<58.1, 58.1–60.5, >60.5); (d) total cholesterol (mg/dl) (<198, 198–230, >230); (e) tryglicerides (mg/dl) (<88, 88–131, >131); (f) uric acid (mg/dl) (<4.2, 4.2–5.4, >5.4); (g) α-tocopherol (μmol/l) (<19.5, 19.5–22.63, >22.63); (h) plasma creatinine (mg/dl) (<0.83, 0.83–0.96, >0.96); (i) glycemia (mg/dl) (<88.0, 88.0–92.0, >92.0); (j) lymphocytes (number) (<3.13, 3.13–4.02, >4.02); (k) neutrophiles (number) (<1.57, 1.57–2.07, >2.07). Cytokines were categorized according to tertiles of distribution: (a) IL-1 Ra (pg/ml) (<105.29, 105.29–159.92, >159.92); (b) IL-1β (pg/ml) (<0.09, 0.09–0.15, >0.15); c) IL-6 (pg/ml) (<0.87, 0.87–1.70, >1.70); (d) IL-6 R (ng/ml) (<74.96, 74.96–110.15, >110.15); (e) TNF-α (pg/ml) (<3.57, 3.57–6.70, >6.70); (f) since a large number of subjects had undetectable levels of IL-10, IL-10 was clustered in two groups (greater or equal to 0.1 pg/ml versus undetectable).

2.7. Statistical analysis

Differences among groups in nerves conduction velocity were evaluated by analysis of variance with generalized linear model (PROC GLM). All preliminary analyses were adjusted for age, sex and age × sex interaction, under the assumption that the effect of age on nerve conduction velocity was different in men and women. The linearity of the age-associated decline in nerve conduction velocity was verified in subsequent regression models by testing whether the combined effect of age and age-squared significantly improved the prediction of nerve conduction velocity, compared to age only.

Variables that in the univariate analyses were associated with nerve conduction velocity with a *p* value of 0.10 or less were entered in a linear regression model (PROC REG) predicting nerve conduction velocity.

A *p* value less or equal to 0.05 was used to establish statistical significance. Analysis for outliers and for leverage was also conducted.

All analyses were performed using SAS release 8.2.

3. Results

Of the 1292 subjects enrolled in the study, 573 (44.3%) were males and 719 (55.7%) females. Average NCV declined with age in both men and women, and in each age-group NCV was higher in women than in men (Fig. 2). In fact, no significant differences could be found between men and women until the 50–54 years group; after this age, the sex specific trajectories show different slopes, with men having a lower NCV, than women, in the 90+ years cluster.

There was a significant age × sex interaction in the model predicting NCV, indicating that the role of age associated decline in NCV was significant steeper in men than in women.

The age-associated decline in NCV was linear. In fact, introducing a quadratic term for age in the regression model did not significantly improve the prediction of NCV ($p = 0.49$ for age as quadratic term).

Of the entire population enrolled in the study, 304 (23.5%) reported to be teetotalers.

After adjusting for age and sex, participants who reported to be teetotalers and those who reported drinking alcohol had similar NCV (44.6 ± 3.8 versus 44.7 ± 4.0; $F = 0.60$; $p = 0.44$). Similarly, participants who never smoked had NCV comparable to current or former smokers ($F = 3.6$; $p = 0.07$).

Independent of age and sex, diabetes and presence of cognitive impairment were both associated with a lower NCV (Table 2). Among the cytokines considered, NCV was significantly lower in participants in the upper IL-6 and sIL-6R tertiles.

After adjustment for age and gender, difference of NCV across IL-6 tertiles were no longer statistically significant, while differences across sIL-6R tertiles were only slightly reduced and still statistically significant (Table 3).

Independent of age and sex, participants in the highest tertiles of neutrophils number, α1 and α2-globulin serum protein, had significant lower NCV than those in the lowest tertiles. Conversely, higher total cholesterol, higher plasma α-tocopherol, higher uric acid and higher lymphocyte number were independently associated with higher NCV (Table 4).

3.1. Linear regression analysis

Table 5 reports the results of the multivariable analysis. As already mentioned, in the saturated model were forced all variables that in the age and sex adjusted analysis were associated with the outcome with a p value <0.10.

The first model (Table 5, right panel, "For trend values") showed that diabetes, cognitive impairment, sIL-6R, plasma level of α-tocopherol and uric acid were significant independent predictors of NCV, besides age, sex, their interaction, height, lymphocytes, neutrophils number, α1 and α2-globulin serum protein and serum creatinina. Participants with intermediate (26–32.9 μmol/l) and high level (>32.9 μmol/l) of α-tocopherol and high level of uric acid (5.4 mg/dl) had significantly higher in NVC compared to those in the lowest tertile. Conversely, for sIL-6R participants in the highest tertile (110 ng/ml) had a lower NCV whereas no differences was found comparing participants with intermediate and low sIL-6R levels (Table 5 left panel). The R^2 of the model was 0.30; with age, sex and their interaction alone accounting for 20% of NCV variance.

4. Discussion

Using data collected in a large population-based sample of persons over a wide age range, we found that, NCV decline linearly with age both in men and in women. Diabetes, cognitive impairment, high sIL-6R, uric acid and low Vitamin E levels, were all associated with lower NCV, independent of age, sex, their interaction, height, α1 and α2-globulin serum protein, serum creatinine, lymphocytes and neutrophils.

The progressive decline of NCV with age has been already reported in the literature, but the association has been described either as linear or quadratic [4,24]. Our findings are in agreement with the notion that NCV declines linearly with age since we were unable to detect any departure from a linear decline across the entire life span.

Impaired conduction velocity with aging may be attributed to structural changes, including a marked fiber loss, involving both unmyelinated axons and myelinated fibers, and the development of abnormalities in the surviving nerve fibers. Specifically, macrophages and mast cells increase substantially in the endoneurium; Schwann cells develop bulb-like extrusion and collagen pockets. Changes in peripheral nerve metabolism habe are also been described [33].

Also progressive dysregulation of glucose metabolism that often occur in older individuals determine a loss of efficiency in the Na/K-ATPase activity, a shift to anaerobic glycolysis, accumulation of polyols, increased protein glycation also contribute to the decline in NCV in

aging individuals [12]. Lastly, in older persons, a reduction in nerve blood flow can result in the paradigmatic picture of neuropathy [33].

Women had significantly faster NCV than men across all age groups considered in this study. Estrogens are known to exert a strong influence on many neurological structures [18], both during development and adult life. Although very little is known about the effects of estrogens on the peripheral nervous system, estrogen receptors have been found in motoneurons [20]. The relative estrogen deficiency that occurs in the postmenopausal period may affect the rate of NCV decline in aging women compared to men.

Diabetes and cognitive impairment were the only diseases independently associated with nerve conduction velocity. The impairment of nerve conduction velocity is among the earliest abnormalities detected in the early stage of diabetes and is directly correlated with the disease duration and the degree of metabolic control. Although several mechanism have been proposed to explain the genesis of diabetic neuropathy increased oxidative stress is considered to play a preeminent role [28]. In addition chronic hyperglycemia causes increased production of reactive oxygen species (ROS) leading to production of superoxide and hydroxyl radicals, consequently, the oxidation of cell structure may induce functional and structural changes [30].

There are many evidences that the oxidative stress and the consequent accumulation of free radicals could be also a causative factor in the genesis of the Alzheimer disease [32,34]. Vitamin E is the major chain-breaking antioxidant present in biological membranes and fluids and an essential factor for the development of the normal structure and function of the human nervous system and the maintenance of their integrity over the life span. Vitamin E deficiency causes symptoms of cerebellar dysfunction and peripheral neuropathy that is partially reversible by Vitamin E supplementation administered at an early stage. Interestingly, the administration of Vitamin E in an early phase of diabetes improves peripheral nerve function in diabetic rats [5] and prevents the development of nerve conduction deficits in young streptozotocin-diabetic rats [15].

There is also evidence that the administration of Vitamin E in type 2 diabetic patients slows-down the progression of peripheral neuropathy [28]. Additionally, the administration of Vitamin E reduces nerve malondialdehyde levels [29] suggesting that its protective action may be mediated, at least in part, by its antioxidant activity against free radical induced damage.

Both pre-clinical and clinical data have shown a clear connection between oxidative stress and the production of inflammatory mediators. Antioxidants, such as Vitamin E reduce the production of IL-1β, IL-8 and TNF-α by leukocytes [31].

There is also evidence that older person are often affected by a mild pro-inflammatory state that has been attributed to primary dysregulation of the immune response, and might contribute to accelerate the physiological decline associated with aging [9]. Inflammatory states are pleomorphic and involve a variety of cell types, including not only neutrophils but also macrophages and lymphocytes. These cells may act by releasing a wide range of signaling molecules, including growth factors, cytokines and chemokines. Many of these molecules, and in particular the IL-6 family cytokines through a receptor complex exert important biological effect on the nervous system cells.

The delivery of IL-6 after an insult, in the central and in the peripheral nervous system promotes neuronal survival and outgrowth, attenuates motor deficits and may accelerate nerve regeneration.

Recent evidences suggest that neuronal cells require sIL-6R to be responsive to IL-6 [11,14, 21].

For example, in pre-clinical models of axonal damage, the addition of IL-6 and sIl-6R enhances the speed of axonal regeneration, while the addition of IL-6 or sIl-6R alone have virtually no effects.

It has also been demonstrated that high levels of proinflammatory cytokines and high oxidative stress are associated with neuronal apoptosis. Thus, it is conceivable that a dysregulation of the inflammatory pathway and the excessive production of ROS may negative influence neuronal function, which results clinically in lower NCV.

The main limitation of this study concerns the cross-sectional design, that does not enable us to establish a direct causal effect relationship. Additionally, because of the epidemiological nature of this study, we were able to collect only one measure of motor conduction velocity so we cannot exclude that variable associated with sensitive nerve conduction could have been different.

Our results support the hypothesis that inflammation and increased (impaired) oxidative stress accelerate the decline of function in the peripheral nervous system that is often observed during the aging process.

This hypothesis should be further confirmed in epidemiological longitudinal studies whether the chronic administration of antioxidants or anti-inflammatory agents slow-down the neuronal age-associated decline in NCV should be tested in randomized controlled trials.

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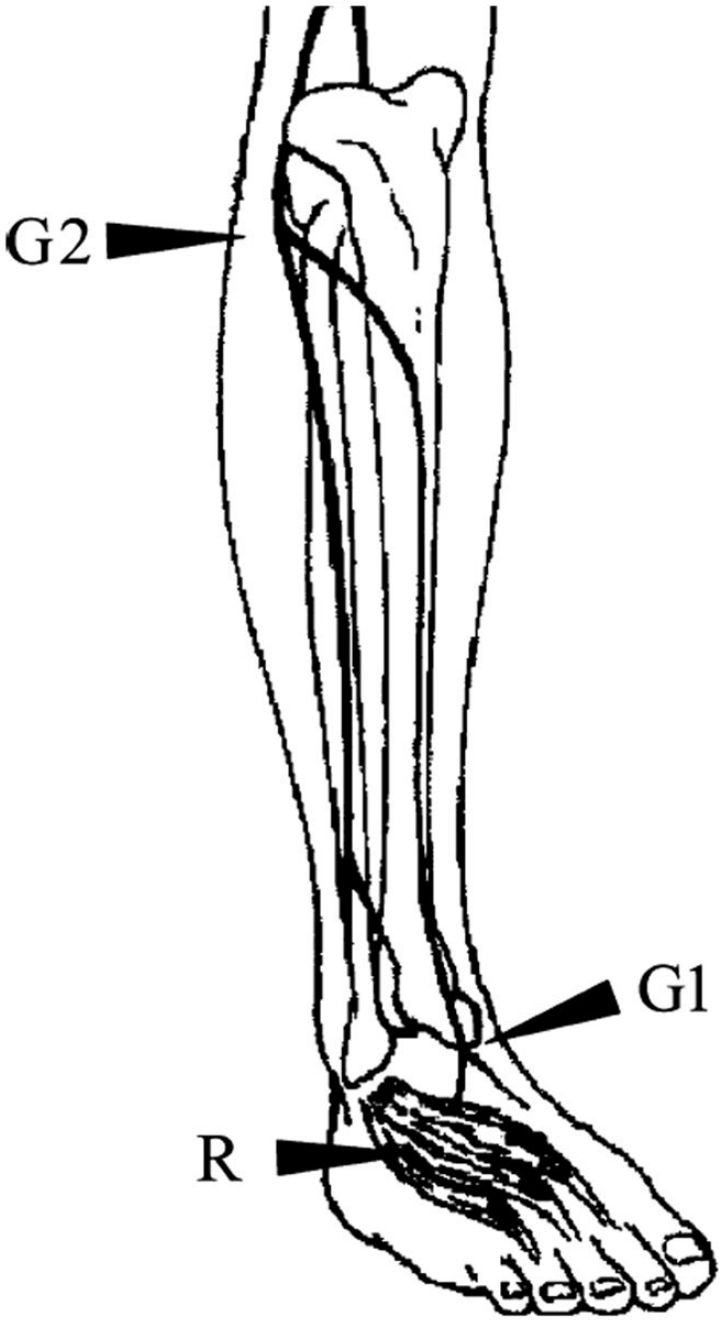

Fig. 1.
Motor nerve conduction of the peroneal nerve. For recording (R), a surface electrode is placed on the extensor digitorum brevis muscle. For stimulation, the peroneal nerve is stimulated proximally at the fibular head (G2) and distally over the anterior ankle (G1) [13].

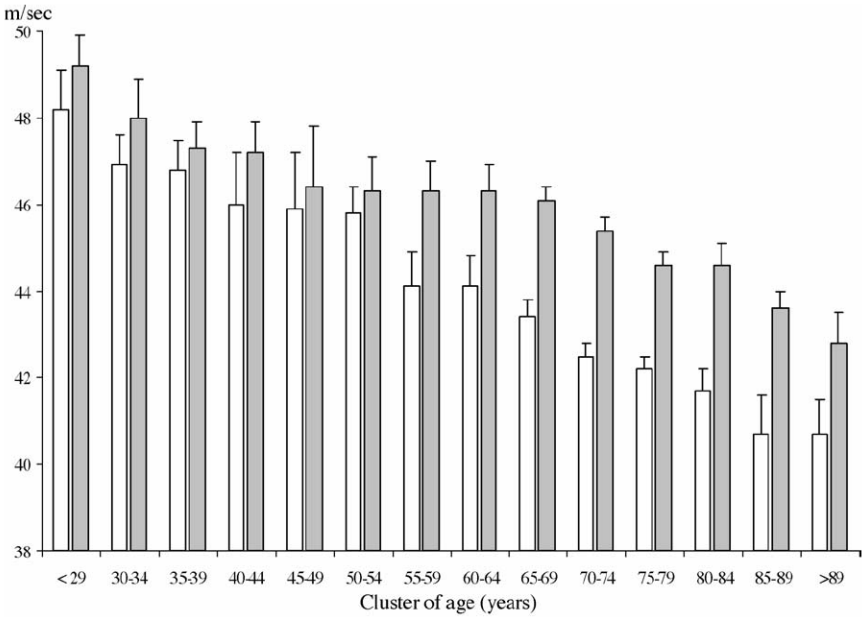

Fig. 2.

Nerve conduction velocity according to cluster of age and sex. The age-associated decline was linear. The interaction between cluster of age and sex was also significant. Nerve conduction velocity was measured in m/s. Age was clustered in groups of 5 year. White column rappresent men and grey histograms women.

Table 1

Participation rate in the different sections of the InCHIANTI baseline evaluation, according to sex and age group

	Greve	Bagno	Total	Interview		EPIC		Blood sample		Medical examination		Functional evaluation		pQTC	
	n	*n*	*n*	*n*	%	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]	*n*	%[a]
Men															
<65 years	90	86	176	143	81	142	99	136	95	134	94	134	94	131	92
65–69 years	76	83	159	142	89	142	100	136	96	136	96	136	96	129	91
70–74 years	86	72	158	142	90	141	99	134	94	132	93	130	92	127	89
75–79 years	55	48	103	102	99	102	100	96	94	94	92	94	92	91	89
80–84 years	26	28	54	49	91	47	96	43	88	41	84	40	82	37	76
85–89 years	21	23	44	39	89	39	100	32	82	33	85	32	82	24	62
90+ years	15	20	35	23	66	21	91	18	78	18	78	18	78	13	57
Total men	369	360	729	640	88	634	99	595	93	588	92	584	91	552	86
Women															
<65 years	97	94	191	155	81	154	99	152	98	148	95	148	95	144	93
65–69 years	104	86	190	171	90	169	99	160	94	157	92	157	92	152	89
70–74 years	83	85	168	155	92	155	100	145	94	144	93	144	93	134	86
75–79 years	73	86	159	131	82	131	100	124	95	121	92	120	92	117	89
80–84 years	42	48	90	85	94	85	100	73	86	71	84	70	82	67	79
85–89 years	41	45	86	72	84	72	100	57	79	54	75	53	74	45	63
90+ years	24	29	53	44	83	44	100	37	84	34	77	32	73	21	48
Total women	464	473	937	813	87	810	100	748	92	729	90	724	89	680	84
Total	833	833	1666	1453	87	1444	99	1343	93	1317	91	1308	90	1232	85

[a]Percentage of those interviewed.

Table 2

NCV in the InCHIANTI participants according to disease status

	Disease absent		Disease present		Unadjusted		Adjusted	
	Number	**Mean ± S.D.**	**Number**	**Mean ± S.D.**	***F***	***p***	F^a	p^a
Diabetes	1098	44.8 ± 3.9	108	41.9 ± 3.8	50.8	<0.001	38.0	<0.001
Cognitive impairment[b]	1155	44.7 ± 4.0	51	42.2 ± 5.0	18.9	<0.001	7.3	0.007
Stroke	1141	44.6 ± 4.0	65	42.1 ± 4.3	21.5	<0.001	7.0	0.008
Peripheral arterial diseases	1149	44.6 ± 4.0	57	41.7 ± 3.7	29.8	<0.001	9.3	0.002
Congestive heart failure	1166	44.6 ± 4.0	40	42.9 ± 4.1	7.2	0.007	1.0	0.31
Acute myocardial infarction	1159	44.6 ± 4.1	47	43.4 ± 3.6	2.6	0.11	0.1	0.75
Hypertension	703	44.7 ± 4.2	503	44.2 ± 3.7	6.0	0.01	0.3	0.57
Angina	1162	44.6 ± 4.0	44	43.7 ± 3.9	2.2	0.13	0.4	0.52
Parkinson	1195	44.5 ± 4.0	11	42.7 ± 8.9	2.2	0.14	0.4	0.53
Hip fracture	1180	44.6 ± 4.0	26	43.6 ± 3.9	1.9	0.17	1.4	0.23
Deep venous thrombosis	1174	44.5 ± 4.0	32	44.6 ± 3.0	0.0	0.83	2.0	0.16

Comparisons between groups were performed by general linear model.

[a] *F* and *p* value adjusted for age (years), sex and their interaction.

[b] MMSE less or equal 19 corrected for school level and age.

Table 3

Peripheral nervous conduction in the InCHIANTI participants according to inflammatory markers tertiles

	Cytokines serum level tertiles[a]						Unadjusted		Adjusted	
	Lower		Intermediate		Higher		*F*	*p*	F^b	p^b
	Number	Mean ± S.D.	Number	Mean ± S.D.	Number	Mean ± S.D.				
IL-1β (pg/ml)	396	44.4 ± 4.0	381	44.5 ± 3.9	429	44.7 ± 4.3	1.96	0.16	2.34	0.13
sIL-6R (ng/ml)	400	44.9 ± 4.1	397	44.7 ± 4.0	409	44.0 ± 4.0	8.6	0.003	4.10	0.04
IL-6 (pg/ml)	412	45.4 ± 4.0	400	44.5 ± 3.9	394	43.7 ± 4.0	39.8	0.001	1.10	0.30
IL-1Ra (pg/ml)	413	44.6 ± 4.1	393	44.7 ± 3.9	400	44.2 ± 4.1	1.15	0.28	0.15	0.70
TNF-α (pg/ml)	414	44.6 ± 3.9	377	44.3 ± 4.0	416	44.6 ± 4.3	0.01	0.91	0.02	0.90

Comparisons between tertiles were performed by general linear model.

[a] Lower, intermediate and higher tertiles were calculated on the entire sample (1292 subjects enrolled in the study) and were referred to the distribution of the single cytokine. Mean ± S.D. of nerve conduction velocity were reported according to tertiles of distribution of every Cytokine.

[b] *F* and *p* value adjusted for age (years), sex and their interaction.

Table 4

Peripheral nervous conduction in the InCHIANTI participants according to tertiles of laboratory parameters

	Tertiles[a]						Unadjusted		Adjusted	
	Lower		Intermediate		Higher		*F*	*p*	*F*[b]	*p*[b]
	Number	Mean ± S.D.	Number	Mean ± S.D.	Number	Mean ± S.D.				
α1 globulin (% total protein)	487	45.2 ± 3.9	366	44.4 ± 3.9	352	43.7 ± 4.3	28.9	<0.001	15.8	<0.001
α2 globulin (% total protein)	398	44.7 ± 4.0	407	44.8 ± 4.0	401	44.1 ± 4.1	4.7	0.03	4.5	0.03
Albumin (% total protein)	407	44.2 ± 4.0	366	44.3 ± 4.2	432	45.0 ± 3.9	7.7	0.006	0.01	0.95
Total cholesterol (mg/dl)	397	44.1 ± 4.4	389	44.4 ± 4.0	420	44.9 ± 3.7	8.3	0.004	10.6	0.001
Tryglicerides (mg/dL)	399	44.6 ± 4.2	399	44.3 ± 4.0	408	44.6 ± 3.9	1.1	0.30	2.0	0.16
α-Tocopherol (μmol/l)	382	44.0 ± 4.4	400	44.7 ± 3.9	424	44.8 ± 3.8	8.6	0.003	9.3	0.002
Lymphocytes (number)	386	44.1 ± 4.0	408	44.3 ± 3.9	412	45.1 ± 4.2	14.2	<0.001	6.0	0.01
Neutrophils (number)	410	44.9 ± 3.8	400	44.7 ± 3.8	396	43.9 ± 4.4	13.1	<0.001	4.3	0.04
Uric acid (mg/dl)	343	45.0 ± 3.9	466	44.5 ± 4.1	397	44.2 ± 4.1	6.7	0.009	6.3	0.01
Creatinine (mg/dl)	405	45.5 ± 3.9	384	44.4 ± 3.9	417	43.6 ± 4.1	47.2	<0.001	3.1	0.07
Glucose (mg/dl)	376	45.1 ± 4.2	402	44.9 ± 3.9	428	43.6 ± 4.0	25.0	<0.001	1.5	0.22

Comparisons between tertiles were performed by general linear model.

[a] Lower, intermediate and higher tertiles were calculated on the entire sample (1292 subjects enrolled in the study) and were referred to the distribution of the single laboratory parameters. Mean ± S.D. of nerve conduction velocity were reported according to tertiles of distribution of laboratory parameters.

[b] *F* and *p* value adjusted for age (decades) and sex.

Table 5

Multiple linear regression; factors associated with peripheral nervous conduction in the InCHIANTI participants

	B	SEβ	*t*	*P*	Test for trend	
					t	*p*
Diabetes	−2.15	0.35	−6.24	<0.001	−6.22	<0.001
Cognitive impairment[a]	1.07	0.54	2.00	0.04	2.77	0.005
sIl6R (tertiles)					−1.99	0.05
<74.96 (ng/ml)		Reference group				
74.96–110.15 (ng/ml)	0.17	0.24	0.72	0.47		
>110.15 (ng/ml)	−0.47	0.23	−2.00	0.04		
α-Tocopherol (tertiles)					2.20	0.03
<26 (μmol/l)		Reference group				
26–32.9 (μmol/l)	0.46	0.23	1.88	0.05		
>32.9 (μmol/l)	0.49	0.24	1.98	0.04		
Uric acid (tertiles)					2.57	0.01
<4.2 (mg/dl)		Reference group				
4.2–5.4 (mg/dl)	0.43	0.24	1.67	0.09		
>5.4 (mg/dl)	0.73	0.28	2.54	0.01		

The model shown in this table is adjusted for age, gender, their interaction, lipid levels, serum creatinine, height, lymphocytes (number), neutrophils (number), α1 and α2 proteic fractions.

[a]Cognitive impairment was defined as a MMSE score lower or equal to 19 corrected for education and age.