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# Carotid ultrasound examination as an aging and disability marker

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**Aim:** To explore the usefulness of carotid ultrasound examination as a marker of aging and predictor of disability among older people.

**Methods:** Carotid ultrasound, measuring carotid intima media thickness (CIMT) and recording the presence of plaques, was carried out in 152 adults aged 29–59 years (47 women) and in 107 older adults aged 61–88 years (86 women). In all, clinical routine laboratory parameters and lymphocyte telomere length as T/S ratio were measured. Among older adults, 12-min walk, timed up and go, hand grip and quadriceps strength were determined.

**Results:** CIMT was significantly higher among older people and T/S ratio was significantly higher in young women. Carotid plaques were found in one adult and 17 older people. A multiple regression analysis accepted age, systolic blood pressure and T/S ratios as independent predictors of CIMT ($R^2 = 0.51$). Among older people, a logistic regression accepted age and the presence of carotid plaques as significant predictors of a 12-min walk speed below 1 m/s.

**Conclusions:** An abnormal 12-min walk as an indicator of functional decline among older people is associated with the presence of carotid artery plaques. CIMT is independently associated with age. **Geriatr Gerontol Int 2014; 14: 710–715.**

**Keywords:** aging, carotid intima media thickness, functional decline.

## Introduction

Carotid intima media thickness (CIMT) measured by ultrasound is closely related to age,[1] independent of other factors affecting it.[2] It is also negatively associated with leukocyte telomere length.[3] Thickening of the intima media has been considered as a physiological effect of aging, because of its linear relationship with age.[4] This measurement has the advantage of being non-invasive, simple and reproducible. The use of border detection software increases its accuracy and reproducibility.[5,6] However, there is no evidence that the increase in CIMT parallels functional deterioration in older adults. Therefore, CIMT could become a good aging parameter that deserves further exploration.

Thus, the aim of the present study was to evaluate the performance of CIMT and telomere length, expressed as T/S ratio, as markers of aging or functionality in healthy participants with a wide age range.

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## Methods

Healthy adults of both sexes were invited to participate in the present investigation. We excluded participants with undernutrition defined as a body mass index below 18 kg/m$^2$, life-threatening diseases, such as cancer, autoimmune diseases, kidney, liver or cardiac failure, with decompensated diabetes mellitus, who were taking medications that could affect muscle function or modify body composition, such as systemic adrenal steroids or anabolic drugs, or who were unable to understand the informed consent because of cognitive impairment. According to age, participants aged 30–59 years were classified as adults, and participants aged 60 years or older were classified as older people. All participants signed a written informed consent, and the study was approved by the ethics committee at the Institute of Nutrition and Food Technology.

All participants underwent the following evaluations:

1. A complete medical examination, including medical history and physical examination, including blood pressure measurement.
2. Anthropometry, measuring weight, height, waist and hip circumference.
3. Withdrawal of a fasting blood sample to measure routine blood chemistry, and to obtain peripheral

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blood monocyte cells (PBMC) to extract genomic DNA.

4 Body composition analysis using General Electric iDEXA equipment (Madison, WI, USA).

5 Measurement of CIMT using a General Electric LOGICQe ultrasound device equipped with border detection software. The full length of both common carotid arteries was scanned, and the presence of plaques, defined as a local thickening of the intima ≥1 mm in thickness,[7] was recorded. When plaques were found, the degree of obstruction was analyzed. The recommendations of the American Society of Echocardiography for measurements and plaque screening were followed.[8]

Older participants were subjected to the following additional psychometric and functional assessments:

1 Mini-Mental State Examination,[9] using a validated Spanish version of the test. Those participants who scored less than 22 in the test were excluded from the study, because according to our experience, participants with lower scores are unable to fully understand the written informed consent.

2 Endurance, measured as the distance that participants could walk at a constant pace on a flat surface during 12 min (12-min walk).[10]

3 Timed up and go (TUG), expressed in seconds and fraction (better performance at lower time required).[11]

4 Quadriceps strength using a quadriceps table and a digital force transducer, as previously described,[12] and hand grip strength using a hand grip dynamometer (Therapeutic Instruments, Clifton, NJ, USA). Both measurements were expressed as 1RM in kg.

Laboratory analyses and calculations:

1 Blood glucose, creatinine, total and high-density lipoprotein cholesterol, triacylglycerol, thyroid stimulating hormone, thyroxin and triiodothyronine were measured in a certified clinical laboratory (Vida Integra, Santiago, Chile).

2 Telomere length was measured in genomic DNA extracted from PBMC using a real-time polymerase chain reaction, described by Cawthon *et al*.[13] Results are expressed as a ratio.

3 CIMT was expressed in millimeters. A mean of 100 calculations were used to calculate the average thickness value at each side. The average value of both sides was also calculated. Plaque diameter was not measured.

4 For the 12-min walk, a cut-off point of 1 m/s in gait speed (corresponding total distance walked in 12 min of 720 m) was used to a define a normal or abnormal test.[14]

5 The cut-off point for the TUG was set in 8.1 s, according to reports showing that, above that value, the risk of falls increases significantly[15] among people without disabilities.

### *Statistical analysis*

Data was analyzed using Stata 12 for Windows (Statacorp, College Station, TC, USA). According to the Shapiro–Wilk test, most parameter values had a non-normal distribution. Therefore, results are expressed as median (range). Groups were compared using the Kruskal–Wallis test. Differences between proportions were assessed using the $\chi^2$-test. Probabilities of 0.05 or less were considered significant. Correlations were calculated using Spearman's correlation coefficient. Multiple stepwise regression equations were used to calculate the associations of multiple independent variables with one dependent variable. Categorical variables, such as sex or test evaluations, were converted to dummies. Logistic regression equations were used to assess the influence of multiple variables on dichotomic dependent variables.

## Results

We studied 259 participants. Of these, 152 were adults (105 women) and 107 were older people (86 women). Demographic, clinical, anthropometric and body composition data of participants are shown on Table 1. As expected, older people had higher systolic blood pressure and lower fat-free mass than young people. The same parameters were lower in women than in men.

Laboratory values and CIMT results are shown in Table 2. Men had higher creatinine and lower high-density lipoprotein cholesterol than women, whereas low-density lipoprotein cholesterol was higher among older people. T/S ratio was higher in young women than in all other groups. CIMT values were significantly higher among older people than in adults. Plaques were found in one adult and 17 older people ($\chi^2 = 19.2$, $P < 0.01$).

Average CIMT values were significantly correlated with age, body mass index, waist and hip circumference, blood pressure, blood glucose, low-density lipoprotein cholesterol, and T/S ratio. A multiple stepwise regression including all variables significantly associated with CIMT in the univariate analysis plus sex as a dummy, accepted age, systolic blood pressure and T/S ratio as significant predictors of average CIMT. (Table 3).

Mini-Mental State Examination scores, muscle strength and functional parameters of older people are shown in Table 4. As expected, men had more quadriceps and hand grip strength than women. No sex differences were observed for TUG and 12-min walk.

A total of 11 older participants had a 12-min walk below the cut-off point (a speed below 1 m/s). Compared with older people with a normal parameter, these were significantly older, had lower quadriceps and hand grip strength, and had a higher frequency of plaques in the common carotid artery (Table 5). A logistic

**Table 1** Demographic, anthropometric and body composition data of participants

| | Adult females ($n$ = 105) | Adult males ($n$ = 47) | Older females ($n$ = 86) | Older males ($n$ = 21) | P¶ |
|---|---|---|---|---|---|
| Age (years) | 42 (29–59) | 42 (29–55) | 73 (61–87) | 72 (61–88) | |
| Weight (kg) | 62.8 (42.2–97.4) | 82 (62.6–104.8) | 63.8 (43.1–99.7) | 75.7 (58.1–96.6) | † |
| Height (cm) | 159 (134–174) | 175 (159–193) | 153 (142–169) | 165 (158–176) | |
| Body mass index (k/m$^2$) | 24.7 (18–44.5) | 26.9 (21.1–36.7) | 27.3 (18.4–41.2) | 28.2 (21.9–33) | ‡ |
| Waist circumference (cm) | 87 (66–123.5) | 95 (83.5–126) | 93 (67–118) | 101 (86–119.5) | |
| Hip circumference (cm) | 99 (84–132) | 101 (92–120) | 101.3 (84–129.5) | 99 (89–117) | |
| Blood pressure (mmHg) | | | | | |
| Systolic | 113 (91–149) | 121 (101–186) | 129.5 (101–200) | 138 (111–197) | |
| Diastolic | 73 (54–100) | 73 (56–107) | 73 (24–110) | 81 (65–102) | § |
| Body composition (DEXA) | | | | | |
| Bone mass (kg) | 2.2 (1.3–3.0) | 3.1 (2.2–4.2) | 2.0 (1.4–2.6) | 2.8 (2.2–3.6) | † |
| Fat mass (kg) | 24.3 (12.3–49.4) | 24.3 (13.2–47.0) | 25.9 (9.7–53.1) | 24.8 (15.4–38.0) | |
| Fat free mass (kg) | 37.6 (25.3–49.0) | 55.7 (43.1–70.3) | 35.3 (24.1–54.0) | 49.3 (40.7–60.6) | † |

Data expressed as median (range). †Women different from men and adults different from older people. ‡Adult females different from all other groups. §Older males different from all other groups. ¶Probability for differences between groups (by Kruskal–Wallis). DEXA, double energy X-ray absorptiometry.

**Table 2** Laboratory values and carotid intima media thickness of participants

| | Adult females ($n$ = 105) | Adult males ($n$ = 47) | Older females ($n$ = 86) | Older males ($n$ = 21) | P‡‡ |
|---|---|---|---|---|---|
| Laboratory values | | | | | |
| Creatinine (mg/dL) | 0.7 (0.5–1) | 1 (0.8–1.5) | 0.8 (0.6–1.8) | 1 (0.7–1.9) | † |
| Blood glucose (mg/dL) | 85 (69–113) | 89 (64–134) | 90.5 (71–162) | 100 (67–135) | ‡ |
| Total cholesterol (mg/dL) | 200 (114–335) | 197 (124–324) | 195 (100–317) | 179 (81–267) | |
| HDL cholesterol (mg/dL) | 59 (29–105) | 42 (27–83) | 59.5 (25–95) | 43 (25–75) | § |
| LDL cholesterol (mg/dL) | 110.4 (49.8–249) | 119.8 (71–225.2) | 161 (90.4–299) | 158.8 (69.4–248.6) | ¶ |
| Triacylglycerol (mg/dL) | 103 (39–350) | 121 (56–707) | 113 (51–515) | 121 (78–240) | |
| T/S ratio | 1.5 (0.32–2.97) | 1.01 (0.31–4.2) | 1.27 (0.3–4.99) | 1.16 (0.29–4.03) | †† |
| Carotid intima media thickness (mm) | | | | | |
| Average left | 0.52 (0.3–0.84) | 0.53 (0.32–0.88) | 0.78 (0.36–1.85) | 0.7 (0.47–1.03) | ¶ |
| Average right | 0.51 (0.24–1) | 0.52 (0.39–1.04) | 0.73 (0.44–1.39) | 0.7 (0.51–1.2) | ¶ |
| Average both sides | 0.52 (0.33–0.85) | 0.52 (0.41–0.88) | 0.74 (0.49–1.33) | 0.67 (0.51–1.02) | ¶ |

Results expressed as median (range). †Women different from men. ‡All groups different. §Adult females different from young males and older males. Young males different from older females. ¶Older people different from adults. ††Adult females different from all other groups. ‡‡Probability for differences between groups (by Kruskal–Wallis). HDL, high-density lipoprotein score; LDL, low-density lipoprotein score.

regression equation including age, quadriceps strength, hand grip strength and the presence of plaques as independent variables, accepted age and the presence of plaques as significant independent predictors of a 12-min walk below the cut-off point. The odds ratio (OR) of the presence of plaques was 7.1 (95% confidence interval [CI] 1.5–33.7).

A total of 21 older participants had a TUG over the cut-off point (8.1 s). Compared with their normal older counterparts, these were significantly older (OR 80, 95% CI 64–87 years and OR 71, 95% CI 61–88 years, respectively, $P < 0.01$) and had a higher frequency of plaques in the common carotid artery (33 and 12%, respectively, $P = 0.02$). The logistic regression did not accept the presence of plaques as a significant predictor of an abnormal TUG.

No other associations between body composition, strength, Mini-Mental State Examination score or other functional parameters and CIMT or T/S ratio were observed.

 

**Table 3** Forward stepwise multiple regression models for average and maximal carotid intima media thickness

| | Coefficient | Standard error | $t$ | $P$ | 95% Confidence intervals | |
|---|---|---|---|---|---|---|
| Dependent variable: average CIMT $R^2 = 0.51$ | | | | | | |
| Age | 0.0065 | 0.0006 | 9.9 | <0.01 | 0.0052 | 0.0078 |
| Systolic blood pressure (mmHg) | 0.0015 | 0.0006 | 2.62 | 0.01 | 0.0004 | 0.0027 |
| T/S ratio | –0.0215 | 0.0119 | –1.81 | 0.072 | –0.0452 | 0.0020 |
| Constant | 0.1341 | 0.0633 | 2.12 | 0.035 | 0.0094 | 0.2589 |

CIMT, carotid intima media thickness.

**Table 4** Mini-Mental State Examination score, muscle strength and functional parameters of older participants

| | Older women ($n$ = 86) | Older men ($n$ = 21) | $P$† |
|---|---|---|---|
| Mini-Mental State Examination score | 29 (22–30) | 29 (27–30) | |
| Muscle strength: | | | |
| Left quadriceps (kg) | 21.8 (6.6–39.3) | 33.4 (13.4–55.1) | <0.01 |
| Right quadriceps (kg) | 24.1 (10.4–38.1) | 32.3 (18.6–57.7) | <0.01 |
| Left handgrip (kg) | 20 (9–30) | 31 (7–42) | <0.01 |
| Right handgrip (kg) | 21 (12–32) | 32 (6–46) | <0.01 |
| Functional parameters: | | | |
| Timed up and go (s) | 6.5 (4.5–15.4) | 6.1 (3.7–10.3) | NS |
| 12-min walk (m) | 938.8 (216.2–1229.2) | 1013.4 (655.2–1363.3) | NS |

Results are expressed as median (range). †Probability for differences between groups (by Kruskal–Wallis).

## Discussion

These results show that CIMT has a strong association with age, whereas T/S ratio is significantly, but weakly, associated with it. More importantly, the detection of plaques by carotid ultrasound was independently associated with a 12-min walk below the cut-off point of normality, marking a relevant functional limitation.

We studied a group of healthy individuals of middle socioeconomic status, mostly sedentary and of both sexes. Among older people, we deliberately did not exclude participants with hypertension and non-insulin dependent diabetes mellitus, as both conditions are highly prevalent in this age group,[16] and their exclusion could have biased our results.

T/S ratio as an indicator of telomere length in PBMC behaved as expected, with lower values among older people than adults. The higher values among young women were not a surprise, as this difference has been previously reported.[17] However, the great problem with this parameter is the dispersion of values. Telomere attrition should be 30–100 bp per year. Therefore, 5 years of change would mean a shortening of 150–500 bp. The interindividual variation of leukocyte telomere length is 5000–10000 bp. Therefore, the variation is more than 10-fold the expected change in a relatively large lapse. These figures show the limitation of leukocyte T/S ratio, using polymerase chain reaction to measure it, as an accurate indicator of aging.[18] The problem of measuring the parameter in a heterogeneous population of cells, such as peripheral leukocytes, should also be tackled.

In contrast, CIMT is a simple measure of arterial damage that is used as a cardiovascular risk factor.[19] However, several reports have minimized the real impact of adding CIMT to the calculation of cardiovascular risk, based on the classical Framingham factors.[20,21] Therefore, there is some skepticism about the real value of CIMT in cardiovascular risk. Nevertheless, all the reports show a strong association of this parameter with age. Furthermore, it is associated with cognitive impairment,[22] cortical atrophy[23] and sarcopenia, which is evaluated by measuring the thigh muscle cross-sectional area.[24] Therefore, CIMT is related to the two most relevant changes that occur during the aging process, namely cognitive impairment and muscle wasting. Furthermore, long term calorie restriction in humans, an intervention that reduces the consequences of aging, significantly reduces CIMT.[25] We have recently observed the same phenomenon after weight reduction in a group of obese patients subjected to bariatric surgery.[26]

The great argument against CIMT as an aging marker is that it only reflects vascular aging, but it is not

**Table 5** Characteristics of participants according to the 12-min walk test

| | Participants with a normal 12-min walk | Participants with a 12-min walk below the cut-off point† | P‡ |
|---|---|---|---|
| Demographic data and Mini-Mental State Examination | | | |
| Female/male (*n*) | 77/20 | 10/1 | NS |
| Age (years) | 71.5 (61–88) | 80 (63–85) | <0.01 |
| Mini-Mental score | 29 (22–30) | 29 (27–30) | NS |
| Body composition | | | |
| Body mass index (kg/m$^2$) | 27.2 (18.4–37.6) | 28.5(21.9–41.2) | NS |
| Total fat free mass (kg) | 36.5 (30.0–60.6) | 33.5 (24.1–54.0) | NS |
| Muscle strength and timed up and go | | | |
| Left quadriceps strength (kg) | 25.1 (9.9–55.2) | 19.9 (6.6–29.0) | 0.04 |
| Right quadriceps strength (kg) | 26.5 (10.4–57.8) | 19.6 (12.0–33.1) | 0.02 |
| Right hand grip strength (kg) | 23 (6–46) | 17 (13–29) | 0.02 |
| Left hand grip strength (kg) | 21 (7–42) | 16 (13–32) | <0.01 |
| Timed up and go (s) | 6.2 (3.7–15.4) | 9.5 (5.3–12.5) | <0.01 |
| Telomere length and vascular parameters | | | |
| Telomere length (T/S ratio) | 1.2 (0.3–5.9) | 1.5 (0.5–4.9) | NS |
| Average carotid intima media thickness (mm) | 0.72 (0.485–1.33) | 0.83 (0.64–1.145) | NS |
| Participants with carotid plaques (%) | 11 | 54 | <0.01 |

†Below 1 m/s. ‡Probability for differences between groups (by Kruskal–Wallis or $\chi^2$ test). NS, not significant.

associated with other events that occur with age. However, the evidence is accumulating that vascular aging is central in age-related decline in organ function. The best example is the pathogenesis of Alzheimer's disease. The former concept that it has no association with vascular abnormalities has been recently challenged.[27] In the present report, we found an association between the 12-min walk and the presence of plaques in the common carotid artery. Although participants with a 12-min walk below the cut-off point were older and had lower muscle strength, the presence of plaques continued to be an independent predictor of an abnormal test in a logistic regression equation. This is other argument to link vascular aging with the loss of muscle function and endurance, which occurs with aging. According to the operational definition, plaque is the ultimate expression of intima thickening, but also a cardiovascular risk factor. It is possible that subtle derangements in muscle perfusion caused by vascular aging, especially during exertion, could lead to functional, and eventually to structural, muscle changes.[28]

The great weakness of the present study was its cross-sectional nature. To reaffirm the value of CIMT as a simple aging marker, a longitudinal study is required to confirm if the decline in motor and cognitive functions that occur with age go along with carotid intima media thickening, and the presence of plaques at baseline and during follow up. If the later parameters result to be predictive of age-related declines in function, they can be used in further studies on aging with greater confidence.

In conclusion, CIMT and the presence of plaques, evaluated by ultrasound, could be associated with age-related decline in functionality, independent of other risk factors, and become a useful marker for aging.

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## Disclosure statement

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