Metabolic syndrome and risk factors for cardiovascular disease: are nonagenarians protected?

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Abstract This study assessed cardiovascular disease risk factors in three groups of human subjects aged 20–34 [young, 20 male (M)/33 female (F)], 60–74 (aged, 29M/29F), and > 90 years (nonagenarian, 47M/50F). Components of the metabolic syndrome, cardiovascular disease, and markers of inflammation and oxidative stress were assessed. Nonagenarians weighed less than the two other groups (\(P<0.001\)); however, there was no difference in percent fat among the three groups. Aged individuals had the highest prevalence of the metabolic syndrome (\(P<0.001\)) according to the Adult Treatment Panel III classification. Both fibrinogen and homocysteine concentrations were significantly higher in the nonagenarians compared to younger groups. However, there were no significant differences between groups in fasting insulin, high sensitive C-reactive protein, and plasminogen activator inhibitor 1 concentrations. There were also no relationships between inflammation/oxidative stress and the metabolic syndrome or cardiovascular disease although nonagenarians appear to be protected from oxidative damage to DNA.

Keywords Aging · Cardiovascular disease · Inflammation · Metabolic syndrome · Oxidative stress

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Introduction

The average life expectancy in the United States is 77 years, and in the western world the chance of living to 100 is 1 in 10,000 (Oppeen and Vaupel 2002; Perls et al. 2002). Individuals achieving this longevity appear to delay or even escape various age-related diseases such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) that typically develop at younger ages (Perls et al. 2000). These individuals may therefore possess genetic and physiological traits that contribute to their exceptional longevity.

Cardiovascular disease is one of the leading causes of death in the westernized world (Kahn et al. 2005). The American Heart Association reports that 84% of individuals diagnosed with CVD are over the age of 65 years. The development of CVD is preceded by traits of the metabolic syndrome, a clustering of risk factors for CVD including obesity, dyslipidemia, hypertension, insulin resistance, and glucose intolerance (Ferrannini et al. 1991; World Health Organization 1999). Some individuals with exceptional longevity are seldom affected by the age-related alterations in CVD risk, although the mechanisms responsible for this younger profile are unknown (Keys et al. 1952; National Health and Nutrition Examination Surveys 1987; Ferrannini et al. 1991; Paolisso et al. 1995, 1997; Roubenoff and Hughes 2000; Ryan 2000; Perls et al. 2002; Barzilai et al. 2003).

Cardiovascular disease is tightly associated with inflammation (Ross 1999). Consistent with this paradigm is the recognition that, in addition to the well-recognized CVD risk factors including lipids and blood pressure, other factors, including hemostasis factors, markers of inflammation and oxidative stress, and acute phase proteins, are of importance. Circulating concentrations of these markers increase with age and are predictive of future CVD events (Kannel et al. 1987; Ridker et al. 1998a, b, 2004; Pearson et al. 2003; Devaraj et al. 2005). Assessment of such factors may help to determine an individual’s “biological age” as opposed to their chronological age. Thus, individuals displaying exceptional longevity may be considered biologically younger than their chronological age and this may contribute to their extended lifespan (Fig. 1). This study tests the hypothesis that nonagenarians have levels of risk factors for CVD comparable to aged subjects and will present with less inflammation and oxidative stress.

Methods

Subjects

The subjects included in this analysis represent a subset of the Louisiana Healthy Aging Study (LHAS) funded as a program project from the National Institute on Aging (Frisard et al. 2007). In this project, extensive data were collected in three groups of individuals aged 20–34, 60–74, and >90 years. Subjects were excluded if they had been diagnosed
with diabetes or had elevated fasting blood sugar (>125 mg/dl), thyroid disease, unstable CVD or mental health problems requiring drug treatment. Nonagenarians were additionally excluded if they had a heart attack or stroke in the 3 months prior to testing, severe high blood pressure, blood vessel aneurysm, taking certain medications used for myasthenia gravis or had uncontrolled asthma, an asthma-like condition or emphysema/chronic obstructive pulmonary disease (COPD). The study was approved by the Institutional Review Boards of the Pennington Biomedical Research Center and the Louisiana State University Health Sciences Center, New Orleans.

Body composition assessment

Weight was measured to ±0.1 kg with an electronic scale (Detecto, Webb City, MO). Height was measured to ±0.5 cm with a wall-mounted stadiometer (Holtain; Crymych, Dyfed, UK), and body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Waist circumference was measured with a standard tape measure while the subject was in the standing position. Whole body composition was estimated by dual-energy X-ray absorptiometry (DXA) (Hologics, QDR 4500A, Bedford, MA).

Blood pressure assessment

Blood pressure was measured in a quiet room with minimal temperature fluctuations following a 5 min rest period. All measurements were taken twice (and averaged) on the participant’s right arm with a manual sphygmomanometer (Baum, Coplaque, NY).

Blood and urinary analyses

Blood samples were drawn in fasting conditions for serum lipids with HDL-C measured by a Sigma Chemical (St. Louis, MO) kit whereas LDL-C was calculated using the Friedewald equation (Friedewald et al. 1972). Glucose was analyzed using a glucose oxidase electrode (Beckman Synchron CX7, Brea, CA), and insulin, high sensitivity C-reactive protein (hsCRP), and homocysteine were measured by immunoassays on a DPC 2000 (Diagnostic Product Corporation, Los Angeles, CA). The HOMA index was calculated as fasting glucose (mmol/L) × fasting insulin (µU/mL)/22.5. Factor VII activity and fibrinogen were measured on an ACL 3000 (Brea, CA). Factor VII was expressed as percent relative to serial dilution of pooled plasma (Clauss 1957). Plasminogen activator inhibitor 1 (PAI-1) was measured by enzyme-linked immunosorbent assay (ELISA) using a kit from American Diagnostica (Stamford, CT). As indices of oxidative stress, urinary isoprostanes were measured using liquid chromatography with mass spectrometry (Davies et al. 2006), serum protein carbonyl content using a modified 2,4-dinitrophenylhydrazine (DNPH) technique (Mates et al. 2000) and DNA fragmentation by single cell gel electrophoresis (Comet and FLARE assay) (Deutsch et al. 2001). Interleukin six (IL-6) was measured by ELISA using a kit from LINSA (St Charles, MO) and run on a Luminex IS 100 (Austin, TX).

Metabolic syndrome classification

Metabolic syndrome was defined according to the Adult Treatment Panel III (ATP III) definition if at least three or more of the following criteria were present: (1) waist circumference >102 cm in men or >88 cm in women, (2) Triglycerides >150 mg/dl or treatment for hypertriglyceridemia, (3) HDL cholesterol <40 mg/dl for men or <50 mg/dl for women or treatment for dyslipidemia, (4) Blood pressure >130/85 mmHg or documented use of antihypertensive therapy, (5) Fasting glucose concentration >100 mg/dl.

Statistical analysis

Data are presented as means ± SEM. Data analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC). Insulin and triglyceride were log transformed to correct for skewing but are presented using untransformed values. Age group and sex effects for subject characteristics were tested using a two-way factorial ANOVA (analysis of variance) and BMI was included as a covariate in the two-way ANOVA model when testing for the cardiovascular risk factors. The statistical significance for all multiple comparisons was adjusted with respect to the Tukey-Kramer method to control for Type I error rate. The level of significance for all statistical tests was set at P<0.05. Pearson correlation coefficients were used to assess relationships between risk factors for CVD, inflammatory markers, and oxidative stress.
Results

A total of 208 participants were enrolled in the present study. The three groups will be referred to as young [20 male (M), 27±1 years; 33 female (F), 28±1 years], aged (29M, 69±1; years 29F, 69±1 years) and nonagenarian (47M, 93±0.3 years; 50F, 92±0.3 years). The young group consisted of 15 white males, 24 white females, 5 black males, and 9 black females. The aged group consisted of 26 white males, 23 white females, 1 black male, 5 black females, 2 “other” males, and 1 other female. The nonagenarian group consisted of 38 white males, 47 white females, 2 black males, 1 black female, 7 other males, and 2 other females. Over the 3 years necessary to recruit the 208 volunteers described, another 177 were recruited in the program. Sixty-five nonagenarians were excluded for the presence of T2DM (N=24) and/or thyroid disorders (N=41) and 75 individuals chose not to come for the 7-h testing at our clinical facility. There were also 25 individuals excluded from the aged group (8 with T2DM, 10 with thyroid disease, and 7 unavailable for 7-h testing) and 12 individuals excluded from the young group (3 with T2DM, 2 with thyroid disease, 1 with pregnancy, and 6 unavailable for 7-h testing). Data for hsCRP, factor VII, fibrinogen, homocysteine, and PAI-1 was available for 200, 198, 199, and 196 subjects, respectively. Data for IL-6, isoprostanes, protein carbonyls, and the comet assays were available for 133, 86, 199, and 189 subjects, respectively.

Body composition

Body composition characteristics of the subjects in the three age groups are displayed in Table 1. In all three age groups, males weighed significantly more than females (P<0.001) and females had higher percent body fat than males (P<0.001). Nonagenarians weighed significantly less than either the young or the aged group (both, P<0.001) and had a significantly lower BMI (both, P<0.001). Thirty percent of the young group, 38% of the aged group, and 8% of the nonagenarians were obese (BMI >30) whereas 30%, 53% and 43% were overweight (30> BMI >25), respectively.

Metabolic syndrome

Thirteen percent of the young group, 63% of the aged group, and 35% of the nonagenarians were classified

| Table 1 | Subject characteristics. Values are presented as means + SEM. Mean values within a row not sharing a common lower case letter are significantly different (P<0.05) for pair-wise comparisons by age group and sex when interaction is significant. Non. Nonagenarian, BMI body mass index, A × S age group by sex interaction |
|---------|-------------------------------------------------------------------------------------------------|
|         | Young (20-34 years) | Young vs Aged (60-74 years) | Aged vs Nonagenarian (>90 years) | Young vs Nonagenarian (>90 years) |
|         | Men | Women | P value | Men | Women | P value | Men | Women | P value | Age group (A) | Sex (S) | A × S |
| Age (years) | 27±1 | 28±1 | | 69±1 | 69±1 | | 93±0.3 | 92±0.3 | | | | |
| Height (cm) | 177±1 | 164±1 | 0.12 | 176±1 | 161±2 | <0.001** | 170±1 | 156±1 | <0.001** | <0.001** | <0.001** | 0.81 |
| Weight (kg) | 90±5 | 74±4 | 0.84 | 90±2 | 78±3 | <0.001** | 72±1 | 60±2 | <0.001** | <0.001** | <0.001** | 0.47 |
| BMI (kg/m²) | 29±2 | 27±1 | 0.30 | 29±1 | 30±1 | <0.001** | 25±0.4 | 25±1 | <0.001** | <0.001** | <0.001** | 0.53 |
| Body fat (%) | 22±2 | 35±1 | b<0.001** | 28±1 c | 41±1 d | 0.02* | 27±1 ce | 35±1 bf | 0.02* | <0.001** | <0.001** | 0.03* |
| Fat Free mass (kg) | 69±2 | 47±1 | b0.22 | 65±1 ac | 46±2 bd | <0.001** | 52±1 e | 38±1 f | <0.001** | <0.001** | <0.001** | 0.006* |
| Fat mass (kg) | 21±3 | 27±2 | 0.01* | 25±2 | 32±2 | <0.001** | 20±1 | 22±1 | 0.19 | <0.001** | <0.001** | 0.21 |

*P<0.05, **P<0.001
as having the metabolic syndrome. Individuals in the aged group had, on average, a significantly greater number of components of metabolic syndrome than the other two groups (Fig. 2, \( P < 0.001 \)). A greater number of nonagenarians were taking medication to treat blood pressure while the same number of individuals in the two older groups was taking medication to treat dyslipidemia. Average values for individual components of the metabolic syndrome for males and females are displayed in Fig. 3. As expected, men had greater waist circumference and lower HDL-C concentrations than females (both, \( P < 0.001 \)). In addition, males also had slightly higher but significant diastolic blood pressure than females (\( P = 0.013 \)). The two older groups had significantly larger waist circumference (\( P < 0.001 \)), higher systolic blood pressure (\( P < 0.001 \)), and higher fasting glucose than the young group (\( P < 0.001 \) for aged vs young, and \( P = 0.002 \) for nonagenarian vs young). Triglycerides were also significantly higher for females in the two older

Fig. 2 Age-related differences in components of the metabolic syndrome. All three groups were significantly different from each other (\( P < 0.001 \)). Numbers of individuals taking medications to treat hypertension and hyperlipidemia are displayed at the bottom.

Fig. 3 Age-related differences in individual components of the metabolic syndrome separated by sex. \( a \) Age group effect, \( b \) sex effect, \( c \) age group \( \times \) sex interaction. Older and oldest females are greater than young.
Table 2  Cardiovascular risk factors. Values are presented as means ± SEM. Means values within a row not sharing a common lower case letter are significantly different (P<0.05) for comparisons of age groups within sex or sexes within age group when interaction is significant. Non, Nonagenarian, A × S age group by sex interaction.

| Young vs aged | Aged vs Non. | Aged vs Non. | Statistical significance of difference between means |
|---------------|-------------|-------------|-----------------------------------------------------|
|                | Age group  | Sex A × S   |                                                     |
|                | Young (20-34 years) |                    |                                                     |
|                | Aged (60-74 years) |                    |                                                     |
|                | Nonagenarian (> 90 years) |                    |                                                     |

|                  | Men          | Women        | P Value       | Men          | Women        | P Value       |
|------------------|--------------|--------------|---------------|--------------|--------------|---------------|
| Insulin (pmol/L) | 81.4±22.6    | 115.8±43.7   | 0.68          | 87.4±11.3    | 93.7±7.36    | 0.83          | 65.7±5.6    | 75.2±6.5    | 0.95          | 0.70          | 0.23          | 0.53          |
| HOMA Index       | 2.78±0.88    | 5.97±3.64    | 0.76          | 2.39±0.23    | 2.71±0.25    | 0.97          | 3.42±0.50    | 3.94±0.49    | 0.87          | 0.77          | 0.25          | 0.48          |
| hsCRP (mg/L)     | 2.5±0.7      | 4.3±1.0      | 0.56          | 4.6±1.1      | 6.3±1.0      | 0.63          | 8.6±2.4      | 3.4±0.5      | 0.11          | 0.13          | 0.74          | 0.03*         |
| Factor VII (%)   | 110.0±5.6    | 109.3±4.5    | 0.08          | 108.3±3.3    | 134.4±4.7    | 0.10          | 107.3±3.5    | 115.4±5.1    | 0.96          | 0.06          | 0.007*        | 0.04*         |
| Fibrinogen       | 9.42±0.55    | 12.51±0.49   | 0.10          | 12.62±2.04   | 11.86±0.56   | <0.001**      | 14.00±0.43   | 14.17±0.46   | <0.001**      | <0.001**      | 0.03*         | 0.001**       |
| PAI-1 (ng/ML)    | 52.7±8.9     | 33.9±7.6     | 0.42          | 65.5±15.6    | 56.3±7.8     | 0.92          | 37.4±9.5     | 45.6±7.0     | 0.62          | 0.43          | 0.45          | 0.35          |
| Homocysteine     | 8.1±0.6      | 6.7±0.3      | 0.06          | 9.9±0.7      | 8.2±0.4      | <0.001**      | 12.8±0.6     | 11.6±0.7     | <0.001**      | <0.001**      | 0.008*        | 0.87          |
| IL-6 (pg/mg)     | 53.6±19.2    | 55.5±21.8    | 0.65          | 77.9±28.1    | 56.7±21.5    | 0.1           | 31.5±8.1     | 46.4±16.7    | 0.48          | 0.11          | 0.97          | 0.62          |
| Isoprostanes     | 36.8±6.7     | 78.0±18.9    | 0.83          | 37.5±3.9     | 64.8±9.8     | 0.68          | 31.7±3.9     | 48.8±6.5     | 0.35          | 0.37          | 0.002*        | 0.55          |
| Protein carbonyls| 1.2±0.2      | 1.1±0.1      | 0.91          | 1.1±0.1      | 1.0±0.1      | 0.99          | 1.0±0.1      | 1.1±0.1      | 0.87          | 0.86          | 0.79          | 0.57          |
| Comet assay (au) | 16.5±0.2     | 16.7±0.1     | 0.21          | 16.8±0.1     | 16.8±0.1     | 0.44          | 16.7±0.1     | 16.8±0.7     | 0.85          | 0.22          | 0.42          | 0.64          |
| Flare assay (au) | 16.9±0.1     | 17.1±0.1     | 0.02*         | 17.4±0.2     | 17.3±0.2     | 0.01*         | 17.0±0.1     | 17.0±0.1     | 0.99          | 0.006*        | 0.89          | 0.44          |

*P<0.05, **P<0.001
groups compared to the young group once the data was log transformed (both \( P < 0.001 \)).

CVD risk factors

CVD risk factors are listed in Table 2. There were no significant differences between groups in fasting insulin, HOMA index, hsCRP, and PAI-1 concentrations nor were there any effects of sex. Males had higher homocysteine plasma concentrations than females (\( P = 0.008 \)). Among the three age groups, the nonagenarians had higher homocysteine plasma concentrations compared to the young and the aged groups (both \( P < 0.001 \)). There were significant age by sex interactions for factor VII activity (\( P = 0.04 \)) and fibrinogen (\( P = 0.001 \)). Females of the aged group had higher factor VII activity than males in the same group (\( P = 0.008 \)). However, factor VII activities for males among the three age groups did not differ. Females in the young group had significantly lower factor VII than females in the aged group (\( P = 0.008 \)). For fibrinogen, there were no sex differences for the aged and nonagenarian groups while the young group showed a significant difference between the sexes (\( P = 0.001 \)). Males of the young group had less fibrinogen than either males in the aged (\( P = 0.002 \)) or males in the nonagenarian (\( P < 0.001 \)) groups. Females in the nonagenarian group had higher fibrinogen than females in the young (\( P = 0.04 \)) and the aged groups (\( P < 0.001 \)).

Markers of inflammation and oxidative stress

Markers of inflammation and oxidative stress are also listed in Table 2. There were no significant differences in urinary isoprostanes, protein carbonyls, or DNA fragmentation as measured by the comet assay between the three groups. Interestingly, females had higher urinary isoprostanes than males (\( P < 0.002 \)). Also, the aged group had significantly higher DNA fragmentation measured by the FLARE assay than both the nonagenarians (\( P = 0.01 \)) and the young individuals (\( P = 0.02 \)). There was a tendency (\( P = 0.10 \)) for higher IL-6 concentrations in the aged compared to the nonagenarians although this did not reach significance. Finally, there were no significant relationships between any markers of inflammation and oxidative stress and any of the components of the metabolic syndrome.

Discussion

These results indicate that risk factors for CVD in nonagenarians are not elevated in proportion to age when compared to individuals aged 60–74. Our findings support the hypothesis that, as previously observed in centenarians, nonagenarians may be biologically young and protected from age-related disease—a factor that may contribute to their longevity. Contrary to our hypothesis, inflammation and oxidative stress were not significantly related to the components of the metabolic syndrome and/or CVD risk factors, although they were not elevated in the nonagenarians compared to the younger individuals. In fact, oxidative damage to DNA was significantly higher and IL-6 tended to be higher in the aged group compared to the nonagenarians and the young individuals. Whether the relatively low levels of inflammation and oxidative stress in nonagenarians confer some resistance to disease remains to be shown.

The aged group had the highest prevalence of the metabolic syndrome. When comparing individual components of the metabolic syndrome in nonagenarians to the aged group, nonagenarians had mostly “better” health values except for systolic blood pressure, which was similar in the two groups. In addition to the widely recognized risk factors for CVD, there are a number of emerging biomarkers also becoming widely accepted, such as hsCRP, fibrinogen, homocysteine, and PAI-1 (Devaraj et al. 2005). In our study, except for fibrinogen and homocysteine concentrations, nonagenarians had comparable levels of CVD markers to those in the younger groups. Specifically, nonagenarians had comparable CRP and PAI-1 concentrations and factor VII activities to the young individuals. PAI-1 is the primary inhibitor of fibrinolysis, and factor VII is a clotting factor that promotes thrombosis. Each of these markers has been demonstrated to be predictive of future coronary events and, in fact, CRP and PAI-1 are better markers of future disease than fibrinogen or homocysteine (Kannel et al. 1987; Ridker et al. 1998b, 2004; Thogersen et al. 1998). Overall, this study supports the hypothesis that nonagenarians may be protected from age-related disease.

Inflammation and oxidative stress are related directly to the progression of atherosclerosis (Wassmann and Nickenig 2003). It was expected that individuals with increased risk for CVD would also have increased inflammatory markers and higher
oxidative damage. Only oxidative damage to DNA was found to be significantly higher in the aged group than either of the other two groups, indicating that nonagenarians may be protected from the age-related increase in DNA damage. However, the finding that inflammation and oxidative damage were not related to cardiovascular risks in the observed population therefore does not support the hypothesis that such factors are implicated in the development of the metabolic syndrome and CVD. On the contrary, the absence of increased inflammation and oxidative stress in the nonagenarians, coupled with levels of CVD markers comparable to younger individuals suggests that nonagenarians may be biologically younger than their chronological age.

Limitations of the current study include potential selection bias and/or birth cohort effects. The prevalence of obesity in the current study was 30, 38, and 8% in the young, aged, and nonagenarians groups, respectively. As such, more individuals from the younger groups may have been “unhealthier” with increased risk of disease thereby diluting differences that would have otherwise been observed. In addition, there were extensive exclusion criteria in order to prevent the confounding effects of existing disease, which may have influenced the study variables. The disadvantage to this is that we may have biased our sample towards a healthier population of older individuals. Consequently, our results should be interpreted with caution.

Conclusion

In conclusion, nonagenarians had less prevalence of the metabolic syndrome and cardiovascular risk factors, which were comparable, if not lower, to those of individuals aged 60–74 years, indicating that their longevity may occur as a result of their biological resistance—probably genetically determined—to disease. Furthermore, the low prevalence of metabolic syndrome in the nonagenarians was not directly related to reduced inflammation and accumulation of oxidative stress.

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