High blood glutathione levels accompany excellent physical and mental health in women ages 60 to 103 years

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Earlier we found a high percentage of subnormal total glutathione (G^T) levels in blood from elderly subjects and patients with chronic diseases. These findings suggested a hypothesis that high levels of G^T in the blood occur in old persons who are in excellent physical and mental health. To this end, we recruited 87 white women who ranged in age from 60 to 103 years and reported that they felt healthy. Their health was verified with physical examinations, clinical chemistry profiles, psychosocial assessments, and blood G^T determinations. This evaluation was performed in three waves over a 5-year period. The values were compared with those from representative individuals in this region and with normal national data. The results verified that these healthy subjects were in top physical and mental health. We also found that subjects of all ages had very high blood G^T levels in waves I and II but only normal levels in wave III. These findings confirm that high blood G^T concentrations and excellent physical and mental health are characteristics of long-lived women. (J Lab Clin Med 2002;140:413-7)

Abbreviations: GSH = glutathione; G^T = total glutathione; HPLC = high-pressure liquid chromatography; SEM = standard error of the mean

For several decades, we have investigated the tissue distribution and function of GSH during the life spans of different organisms, including the mosquito, mouse, rat, and human being.[1-6] Of particular interest was the G^T profile in blood during aging. In the C57BL/6J mouse, the levels in various organs were constant during maturity but declined markedly during senescence. Moreover, the changes in GSH concentration paralleled those in heart, liver, kidney, lung, brain, and spleen during the adult life span. These findings formed our basis for regarding G^T as an accessible index of longevity and also of overall body GSH.

Our current focus has been on GSH and its precursor cysteine in human populations. Initially we sought information on the physical health of elderly people, but we found a paucity of data for individuals more than 65 years old. A notable exception was the New Mexico Aging Process Study,[7] which was primarily focused on nutritional biochemical aspects and less on mental health.

Of special interest was our discovery of blood GSH deficiency in more than half of healthy, elderly subjects 60 to 79 years old, compared with normal levels in the 20- to 39-year-olds who made up the reference group.[5] The criterion for deficiency was a subnormal concentration of erythrocytic GSH, accounting for 99.5% of that in blood.[8] It is therefore a superior index of defi-

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Table I. Major characteristics of healthy 60- to 103-year-old women

Wave	Body-mass index (kg/m^2)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Blood glucose (mg/dL serum)	Cholesterol (mg/dL serum)
I	24.2 ± 0.51 (58)*	145 ± 2.39 (58)	78.8 ± 1.26 (58)	97.9 ± 1.99 (70)	221 ± 3.99 (65)
II	24.5 ± 0.68 (41)	146 ± 3.13 (41)	76.2 ± 1.44 (41)	92.9 ± 1.21 (42)	224 ± 5.48 (42)
III	25.1 ± 0.86 (47)	139 ± 2.76 (50)	77.2 ± 1.45 (50)	106 ± 2.70 (57)†	217 ± 4.24 (56)

*Data expressed as mean ± SEM (*n*).
†Significantly different from wave I ($P < .05$).

ciency compared with plasma, which contains only trace levels of GSH.

In a related experiment, GSH deficiency was found in 36% of newly admitted hospital patients with chronic conditions such as cardiovascular or liver disease and diabetes mellitus.[9] These findings suggested that, in contrast, high G^T levels in elderly subjects would be associated with excellent physical and mental health. Although many reports have considered physical and mental health in elderly subjects, this may be the first to include GSH status.

In this study, we determined blood G^T levels and physical- and mental-health status in healthy elderly women and compared these values with previous findings from representative groups of healthy subjects from this region and with values used nationally.

METHODS

The recruits were from community, university, and personal networks in metropolitan Louisville, Kentucky, and were restricted to white women because of funding limitations. Only women who said they were in good health and without acute medical problems were accepted. Although some individuals said they felt in fine health, their records indicated that they had undergone earlier operations for the removal of cataracts or breast or skin cancers, so they were omitted. Eighty-seven subjects were distributed so that there were 6 to 16 persons in the following age groups: 60 to 69 years, 70 to 79 years, 80 to 89 years, and older than 90 years.

We assessed physical health through examinations performed by an experienced geriatric nurse of the Visiting Nurse Association. Blood samples were collected and analyzed by a national clinical laboratory for a complete blood analysis (red blood cell count, hemoglobin concentration, hematocrit) and a chemistry profile (SMAC 20, or sequential multiple analysis computerized, now called a comprehensive metabolic panel).

GSH concentrations were determined in blood samples that had been collected by venipuncture, immediately chilled on ice, and deproteinized within an hour by the addition of 1 volume of blood to 4 volumes of 5% (wt/vol) metaphosphoric acid. After centrifugation, the acid supernatants were removed and analyzed for G^T as follows: Wave I and wave II utilized the 5,5′-dithio-*bis*-(2-nitrobenzoic acid) and glutathione reductase method of Tietze[10] and Owen and Belcher.[11] Wave III utilized the HPLC–dual electrochemical method of Richie and Lang.[12] The equivalency of these methods has previously been validated.[12] Blood G^T data represent reduced and oxidized forms. The G^T levels of the healthy elderly women were compared to those found earlier in a group of 31 healthy women ages 20 to 39 years with a mean level of 543 ± 55 μg GSH/10^{10} red blood cells (range 433-653).[5]

Mental health was assessed by trained interviewers using questionnaires designed for older individuals, the Belloc health scale,[13] a life-satisfaction scale,[14] and the general well-being scale developed at the National Center for Health Statistics (NCHS) for use in national health examinations,[15] as well as the depression scale used at the Center for Epidemiological Studies.[16] A social-activities and network instrument was developed for persons 60 years and older in Louisville, Jefferson County, Kentucky.[17] This instrument was used to obtain representative reference values typical of residents of metropolitan Louisville and provided information on socioeconomic status and social networks.[18]

The overall procedure was performed in three waves over 5 years. The three waves varied in the number of subjects because of dropouts and replacements, but each subject participated in two or three waves. All women gave their informed consent and had approval of their personal physicians.

Laboratory and clinical data were coded and analyzed statistically with standard tests in Snedecor and Cochran[19] and Lang and Secic.[20] Statistical analyses included correlations, analysis of variance, and Student *t* tests to evaluate significance levels. We considered *P* values of less than .05 statistically significant.

RESULTS

The general physical-health characteristics of the healthy group are presented in Table I. The mean body-mass index in all waves varied from 24 to 25, indicating that the subjects were stable and of normal weight. Their systolic and diastolic blood pressures and average blood glucose values were normal, including the wave III value of 106 mg/dL. The cholesterol levels in all waves were normal at the time of the study but are now considered slightly high. We noted no change between waves I and III. The results of other tests, such as occult blood and pulmonary function, were normal, like those reported for young healthy adults (data not given).

Clinical chemistry and hematology laboratory results are shown in Table II. Profiles were the same for each

Table II. Blood Composition of Healthy 60-103 Year Old Women

Parameter	Wave I	Wave II	Wave III	Normal range*
Red blood cell count ($\times 10^6/\mu L$)	4.60 ± 0.050 (71)†	4.56 ± 0.058 (42)	4.40 ± 0.045 (57)	4.2-5.40
White blood cell count ($\times 10^3/\mu L$)	7.26 ± 0.26 (70)	6.70 ± 0.22 (42)	6.06 ± 0.16 (57)	4.8-10.8
Hemoglobin (g/dL)	14.1 ± 0.14 (70)	14.0 ± 0.16 (42)	13.6 ± 0.16 (56)	12.0-16.0
Hematocrit (%)	40.9 ± 0.44 (70)	41.0 ± 0.49 (42)	39.9 ± 0.43 (57)	37.0-47.0
MCV (fL)	89.6 ± 0.59 (70)	90.5 ± 0.67 (42)	91.3 ± 0.62 (57)	81.0-99.0
MCH (pg)	30.7 ± 0.21 (70)	30.8 ± 0.24 (42)	31.2 ± 0.23 (57)	27.0-31.0
MCHC (g/dL)	33.8 ± 0.11 (70)	34.2 ± 0.061 (42)	34.2 ± 0.082 (57)	33.0-37.0
Blood urea nitrogen (mg/dL)	14.8 ± 0.55 (70)	15.8 ± 0.83 (42)	14.9 ± 0.82 (57)	5-25
Creatinine (mg/dL)	0.96 ± 0.027 (71)	1.00 ± 0.032 (42)	0.98 ± 0.039 (56)	0.7-1.40
Calcium (mg/dL)	9.45 ± 0.047 (68)	9.63 ± 0.061 (42)	9.12 ± 0.044 (54)	8.6-10.8
Phosphorus (mg/dL)	3.80 ± 0.054 (69)	3.56 ± 0.055 (42)	3.27 ± 0.059 (56)	2.5-4.5
Sodium (mEq/L)	141 ± 0.36 (70)	140 ± 0.33 (42)	141 ± 0.35 (56)	135-145
Potassium (mEq/L)	4.44 ± 0.059 (69)	4.54 ± 0.061 (42)	4.07 ± 0.048 (54)	3.3-5.3
Chloride (mEq/L)	102 ± 0.51 (67)	103 ± 0.49 (42)	107 ± 0.45 (57)	96.0-109
Carbon dioxide (mEq/L)	24.2 ± 0.29 (69)	25.7 ± 0.28 (42)	22.4 ± 0.34 (57)	22.0-33.0
Uric acid (mg/dL)	4.85 ± 0.20 (63)	4.81 ± 0.19 (42)	4.87 ± 0.17 (56)	2.2-8.1
Total protein (g/dL)	6.92 ± 0.047 (70)	6.79 ± 0.055 (42)	6.60 ± 0.042 (56)	6.0-8.0
Albumin (g/dL)	4.29 ± 0.033 (69)	4.36 ± 0.041 (42)	3.98 ± 0.029 (56)	3.5-5.5
Total bilirubin (mg/dL)	0.45 ± 0.025 (68)	0.40 ± 0.031 (42)	0.41 ± 0.022 (56)	0.1-1.2
Alkaline phosphatase (U/L)	86.0 ± 3.18 (70)	91.5 ± 3.87 (42)	81.5 ± 2.87 (54)	30-143
ALT (U/L)	20.8 ± 0.85 (65)	19.2 ± 1.01 (42)	17.4 ± 0.83 (56)	0-40
AST (U/L)	28.0 ± 1.17 (70)	17.1 ± 1.26 (42)	15.5 ± 0.99 (55)	0-50

MCV = mean cell volume; *MCH* = mean corpuscular hemoglobin; *MCHC* = mean corpuscular hemoglobin concentration; *ALT* = alanine amino transferase; *AST* = aspartate amino transferase.
*National Health Laboratories Inc.
†Data expressed as mean ± SEM (*n*).

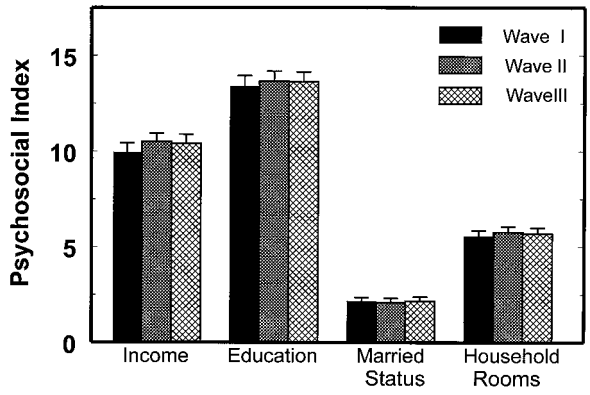

Fig 1. Psychosocial characteristics of healthy elderly women, by waves.

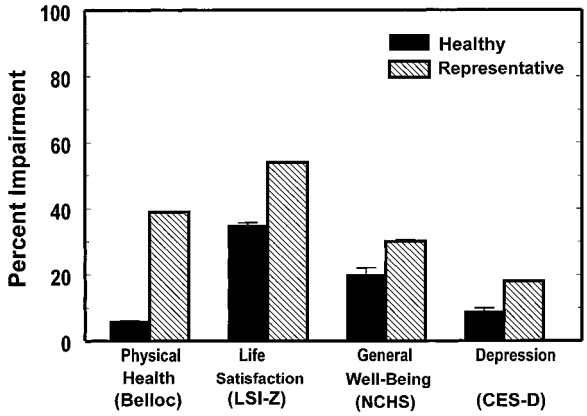

Fig 2. Mental health in the healthy and representative groups. High scores indicate a high degree of pathology; therefore lower scores are "better." All scores from the healthy groups were different from those in the representative group ($P < .001$).

wave, demonstrating the reproducibility and constancy of the laboratory values of the subjects during the 5-year span of this study. In addition, the values were within the normal ranges given in the right column of the table.

The psychosocial aspects of income, education level, marital status, and number of rooms per household are listed for each wave in Figure 1. None of these parameters changed during this study.

The health indexes for physical health, life satisfaction, general well-being, and depression in the healthy and representative groups are shown in Figure 2. The scores for percent impairment were scaled in reverse; therefore a high score indicates a high degree of pathology. The healthy group had significantly lower (and better) scores for every index than the representative subjects ($P < .001$).

Age profiles for these indexes are shown in Figure 3. Again, the healthy women had generally lower scores

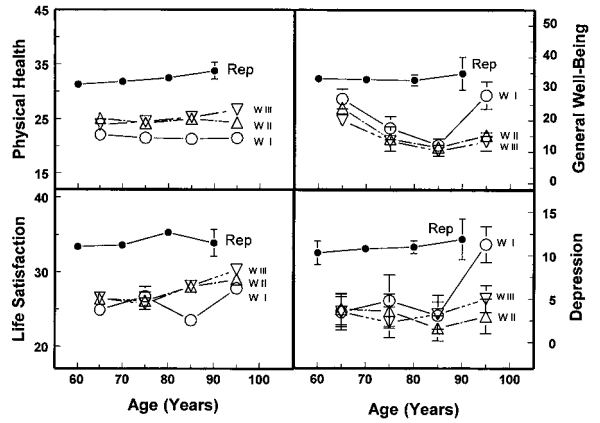

Fig 3. Mental-health indices of healthy and representative groups, by age. *Top curves*, with *solid symbols* and *Rep*, signify the representative group. *Lower curves*, with *open symbols* and *W I*, *W II*, and *W III*, represent waves I, II, and III, respectively. SEM bars for wave I, general well-being, are hidden behind the mean values.

than the women in the representative group. The physical-health and life-satisfaction indexes were near-constant with age. However, two changes were noted in the 95-year group. Wave I values for general well-being and depression were about the same for the healthy and representative groups. These may be areas for further research.

After verifying the excellent physical and mental health of the healthy subjects, we assessed G^T levels (Table III). Mean GSH levels, shown in Table III, were generally high ($P < .05$) compared with the normal range for women reported in an earlier study.[5] Lower levels were found in wave III, in which the values were the same as those in the reference group. The only exception was the 90- to 103-year group, in which G^T levels were statistically higher ($P = .0338$) than in the reference group.

DISCUSSION

Our results confirm the hypothesis that high blood G^T concentrations occur in physically and mentally healthy elderly women. We found several other interesting results.

The major finding was the blood G^T levels of the healthy subjects. They were the highest values we have measured in our experience with several hundred subjects and were in the top portion of the normal range established previously. Furthermore, we found no marked decrease in GSH levels in the 60- to 79-year group, as found earlier.[5] In the previous study, the subjects were assumed to be clinically healthy, but in our experiment, all subjects were rigorously selected and their excellent physical and mental health validated with a battery of tests.

A surprising finding was that the biomedical profiles for the healthy elderly women were the same as those for healthy 20- to 39-year-old adults, the age group used to establish normal adult values in clinical chemistry laboratories. This may be the first time that quantitative objective data have been presented showing that the physical characteristics of healthy women are the same regardless of age.

The evaluation of health status is better determined by physiological than by chronological age. Uniquely, the status of the healthy group was both self-assessed and confirmed with physical examination, laboratory tests, and individual interviews. Other clinical investigations usually assume that the control subjects are in good health, but do not verify this assumption with quantitative tests.

Another unusual aspect of this work was the range of old ages studied: 60 to 103 years. Few clinical studies include subjects beyond 70 to 75 years, yet the current annual increase of life expectancy indicates that information on people of older ages is needed.

This study shows that psychosocial or mental-health status was the same in healthy subjects of different ages and was associated generally with physical health. However, in subjects older than 90 years, some psychosocial aspects, such as general well-being and depression, were subnormal, but physical-health and life-satisfaction indexes remained normal. In this study, there were relatively few persons in the 90-plus-years age group for proper comparison. Do physical and mental health becomes unlinked at very old ages? Does brain function become dissociated from other organ functions during senescence? Further research is needed to answer these questions.

The results of this study are provocative, for these healthy older women were in better condition than the representative subjects, as assessed on the basis of their G^T levels. Most important, the elderly were evaluated in excellent health with the use of objective criteria.

Our original goal was to verify that blood GSH was in high concentration in healthy women older than 60 years. We found that the mean levels of G^T were higher than those in young adults.[5] Therefore the elderly subjects were still in the mature stage, compared with the marked GSH declines of senescence in aging models such as the yellow-fever mosquito (*Aedes aegypti*), the C57BL/6J mouse, and Sprague-Dawley and Lobund rats.[1-6]

Finally, this study was focused on GSH levels and health. It is hoped that idiopathic maladies and diseases will no longer be attributed to aging, a vague and usually ill-defined condition. A more likely possibility is that they are a result of a low GSH level.

Life-span studies are now more feasible, for today

Table III. G^T levels in healthy elderly women compared with normal reference group

Age (yr)	G^T (μg/10^{10} red blood cells)*					
	Wave I	*P* value	**Wave II**	*P* value	**Wave III**	*P* value
60-69	613 ± 22.3 (14)†	<.0017‡	648 ± 21.4 (12)	<.001‡	539 ± 22.4 (12)	.850
70-79	650 ± 14.5 (13)	<.001‡	624 ± 15.7 (16)	<.001‡	504 ± 30.3 (14)	.125
80-89	645 ± 28.9 (11)	<.001‡	682 ± 57.6 (8)	<.001‡	570 ± 52.8 (9)	.420
90-103	744 ± 33.7 (6)	<.001‡	774 ± 61.6 (6)	<.001‡	659 ± 115 (7)	.0338‡

*G^T consists of reduced and oxidized forms.
†Data expressed as mean ± SEM (*n*).
‡$P < .05$ vs normal reference value, 543 ± 9.87 (31).

there exist a number of characterized longitudinal investigations of very old people, such as the centenarian and nun studies such as the New England Centenarian Study siblings.[21] Additional research of such subjects in the future should elucidate the relationships among glutathione, health, and longevity.

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