Plasma total glutathione in humans and its association with demographic and health-related factors

BY ELAINE W. FLAGG[1], RALPH J. COATES[1], DEAN P. JONES[2], J. WILLIAM ELEY[1,3], ELAINE W. GUNTER[4], BETHANY JACKSON[5] AND RAYMOND S. GREENBERG[1]

[1] *Division of Epidemiology, Emory University School of Public Health, Atlanta, Georgia, USA*
[2] *Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia, USA*
[3] *Winship Cancer Center, Emory University School of Medicine, Atlanta, Georgia, USA*
[4] *Nutritional Biochemistry Branch, Division of Environmental Health Laboratory Sciences, Center for Environmental Health and Injury Control, Centers for Disease Control, Atlanta, Georgia, USA*
[5] *Department of Nutrition and Family Studies, Andrews University, Berrien Springs, Michigan, USA*

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The tripeptide glutathione is proposed to be protective against a number of chronic diseases including cardiovascular disease and cancer. However, there have been few studies of plasma glutathione levels in humans and in those studies the numbers of participants have been very small. In an exploratory analysis the determinants of plasma total glutathione (GSH_t) were investigated in a group of 100 volunteers aged 18–61 years in Atlanta, Georgia, USA during June and July 1989. Data on demographic and health-related factors were collected by interview and plasma GSH_t was measured using a recently modified laboratory method. The mean concentration of plasma GSH_t for all 100 participants was 761 μg/l, with a standard deviation of 451 μg/l, a range of 86–2889 μg/l and a median of 649 μg/l. Men had significantly higher levels of plasma GSH_t than women (924 *v.* 692 μg/l; $P = 0{\cdot}006$). Seventh-day Adventists participating in the present study had higher plasma GSH_t levels than other subgroups defined by race and/or religion. Among Seventh-day Adventists consumption of a vegetarian diet was associated with increased plasma GSH_t concentration ($P = 0{\cdot}002$). Plasma GSH_t levels also appeared to vary by race, but relationships with race could not be clearly disassociated from relationships with religion. Among white participants plasma GSH_t concentration decreased with age in women but increased with age in men ($P = 0{\cdot}05$). Few other factors were associated with plasma GSH_t concentration, although use of oral contraceptives ($P = 0{\cdot}10$) was somewhat associated with decreased plasma GSH_t levels. These findings suggest that plasma GSH_t levels may vary with several demographic and health-related attributes and support the need for further research on this potentially important disease-preventive compound.

Plasma glutathione: Disease prevention: Humans

The tripeptide glutathione plays a central role in preventing cellular injury and mutation (Cerutti, 1985; Wefers & Sies, 1988; Stevens & Jones, 1989; Bendich, 1990) and may be a protective factor in cancer and aging (Ames, 1983; Cross *et al.* 1987), cardiovascular disease (Cross *et al.* 1987) and the immune dysfunction of HIV infection (Buhl *et al.* 1989). Although all mammalian cells are capable of glutathione synthesis (Meister, 1989), the liver is the major source of plasma glutathione (Lauterburg *et al.* 1984*a*) and orally administered glutathione increases plasma glutathione concentrations both in animals (Hagen *et al.* 1990; Aw *et al.* 1991) and in humans (Hagen & Jones, 1989). Since epithelial cells are capable of glutathione uptake from plasma (Shan *et al.* 1990; Aw *et al.*

1991), it is possible that people with higher plasma concentrations are at reduced risk of a number of chronic diseases, including epithelial cell cancers. Despite this potential importance there have been few studies characterizing plasma glutathione concentration in healthy humans and sample sizes have been limited to between five and thirty subjects (Owens & Belcher, 1965; Hagenfeldt *et al.* 1978; Wendel & Cikryt, 1980; Chawla *et al.* 1984; Lauterburg *et al.* 1984*b*; Beutler & Gelbart, 1985; Burgunder & Lauterburg, 1986; Buhl *et al.* 1989; Hagen & Jones, 1989). Because of the limited information available and the potential importance of plasma glutathione in disease prevention, we undertook an exploratory study to describe its association with demographic and health-related characteristics in a diverse group of 100 healthy human volunteers.

METHODS

Selection of study participants

Study participants were recruited primarily from among students, faculty and staff of the Emory University School of Medicine, the American Cancer Society and the US Centers for Disease Control. Because Seventh-day Adventists have been found to be at reduced risk of several cancers and other chronic diseases (Phillips, 1975; Phillips *et al.* 1978) and because they tend to differ from the general population with regard to diet and other health-related characteristics (Phillips, 1975; Beeson *et al.* 1989), subjects were also recruited from an Atlanta area Seventh-day Adventist congregation. Current smokers were excluded because smoking appears to modify relationships between serum nutrients and dietary factors (Hunter, 1990). Individuals with major illnesses or taking medication which could potentially affect serum or plasma nutrient levels were also excluded from the study. Medication resulting in exclusion included antibiotics, prednisone, cortisone and medication for conditions such as gout, diabetes, hypertension and high serum cholesterol. Interviewing and blood collection took place between 7 June, 1989 and 31 July, 1989.

A total of 131 eligible people volunteered to participate in the study. Plasma total glutathione (GSH_t) analyses were completed on 123 of these respondents. Twenty-three plasma GSH_t values were excluded due to sample deterioration or suspected haemolysis from visual inspection of the plasma sample. Participants excluded from these analyses did not differ by race, sex or age from those included. The analyses presented here were conducted, therefore, using data from the 100 volunteers with completed interviews and valid plasma GSH_t measurements.

Interview data

After informed consent was obtained, information about health risk factors was collected by personal interview using the Health Habits and History Questionnaire (HHHQ) developed by Block and colleagues (Block, 1989) at the US National Institutes of Health. The HHHQ was developed and validated for epidemiological studies of diet, health-related factors and cancer. Demographic information obtained included the respondent's age, race, sex, marital status, education level and employment status. Data were also collected on a number of health-related characteristics such as former use of cigarettes, current consumption of alcohol, usual amount of sleep and exercise, current use of medication and current or past medical conditions. Trained interviewers used a computerized version of the questionnaire to enter the self-reported responses directly into a personal computer.

Immediately after completion of the interview a non-fasting blood sample was collected from the study participants. Blood collection was obtained by routine venepuncture, using a 19 gauge butterfly needle to avoid haemolysis. Valid sampling for glutathione requires that haemolysis be avoided because erythrocytes contain large amounts of glutathione that does

Table 1. *Demographic characteristics of the study participants*

Demographic characteristic	Men		Women	
	n	% total	*n*	% total
Total	30	100·0	70	100·0
Age category (years)				
18–29	13	43·3	21	30·0
30–39	7	23·3	24	34·3
40–61	10	33·3	25	35·7
Race				
White	23	76·7	49	70·0
Non-white*	7	23·3	21	30·0
Seventh-day Adventist				
No	27	90·0	61	87·1
Yes	3	10·0	9	12·9
Education level				
High School	1	3·3	8	11·4
College	5	16·7	27	38·6
Advanced degree	24	80·0	35	50·0
Employment status				
Employed	21	70·0	57	81·4
Student	7	23·3	11	15·7
Other†	2	6·7	2	2·9

* Includes black (three men, seventeen women), hispanic (one man), and asian (three men, four women) races.
† Includes retired (one man), unemployed (one man, one woman), and homemakers (one woman).

not readily exchange with the extracellular pool and, thus, may not be available for utilization by other tissues (Lash & Jones, 1985).

Laboratory analyses

For the present study we measured plasma GSH_t, a mixture of reduced glutathione and all disulphide forms. To obtain these measurements a 2 ml blood specimen was collected into a tube containing 100 μl heparin (2 mg/ml) in normal saline (9 g NaCl/l) to prevent coagulation and 400 μl 10 mM-1,10-phenanthroline in normal saline to prevent auto-oxidation. This tube was immediately centrifuged in a Fisher microcentrifuge for 0·5 min to obtain plasma. 5-Sulphosalicylic acid (100 mg/ml; 250 μl) was added to 500 μl of the plasma supernatant fraction. The tube was then placed on dry ice for transport to the laboratory for analysis.

In the laboratory 50 μl 10 M-NaOH was added to neutralize the acid and 100 μl 10 mM-dithiothreitol in 0·1 M-NaH_2PO_4 (pH 6·0) was added to release glutathione from all disulphide forms. After 15 min of incubation at room temperature, 500 μl of a solution of 2 parts methanol and 1 part chloroform was added to remove lipids. The tubes were centrifuged for 2 min and the aqueous phase was drawn off and treated with 500 μl trichloracetic acid (300 mg/ml) to remove the protein. Following centrifugation for 2 min the supernatant fraction was drawn off. Iodoacetic acid (40 μM; 100 μl) and 1 M-KOH–K_2CO_3 (pH 8·0; 150 μl) were added to 500 μl supernatant fraction, which was than incubated for 1 h at room temperature. 1-Fluoro-2,4-dinitrobenzene (15 μl/ml; 500 μl) in absolute ethanol and 1 M-KOH–K_2CO_3 (pH 8·0; 50 μl) were added to the supernatant fraction, which was incubated for 4 h at room temperature. The glutathione derivative was determined by the HPLC method of Reed *et al.* (1980), with minor modifications by Lash & Jones (1985).

Statistical analyses

The overall distribution of plasma GSH_t was characterized in the study participants by calculating the mean, standard deviation, median and range of values for all 100 subjects. To determine whether the plasma GSH_t concentration was normally distributed the data were plotted in a normal probability plot (Neter *et al.* 1985) and a Kolmogorov-Smirnov goodness of fit D statistic was calculated (Zar, 1984). Because the distribution of plasma GSH_t concentration was skewed the values were $\log_e$-transformed to produce normally distributed data for further analyses.

In order to compare plasma GSH_t concentrations among study subgroups defined by the demographic and health-related characteristics of interest, mean values for the subgroups were calculated using $\log_e$-transformed data. Because the study participants were volunteers, and, therefore, cannot be assumed to represent the population from which they were drawn, statistical tests and 95% confidence intervals for subgroup means were based on the mean square error (MSE) from analysis of variance (ANOVA) for unbalanced data, which gives equal importance to all analysis subgroups (Neter *et al.* 1985). Geometric means for these subgroups and associated upper and lower 95% confidence bounds were calculated by taking the antilogs of $\log_e$-transformed data (Flanders *et al.* 1992).

The sex- and/or age-adjusted associations between demographic and health-related characteristics and plasma GSH_t concentration were examined using linear-regression models which incorporated indicator variables for the factors of interest as well as sex and/or age indicators (Neter *et al.* 1985). Factor level (i.e. subgroup) means with their standard errors were calculated using the variance–covariance matrix values and beta parameter estimates from the best fitting linear-regression model and substituting the entire group's means for sex and/or age variables in the X matrix of constants. Confidence intervals (95%) about adjusted factor level means were calculated using adjusted standard errors.

Statistical significances of differences between geometric means were determined by taking differences between means from $\log_e$-transformed data and calculating the standard errors of the differences based on the MSE. Subgroup comparisons which were adjusted for sex and/or age category were similarly obtained using adjusted means and adjusted standard errors. Bonferroni and Tukey procedures were used to correct for multiple comparisons (Neter *et al.* 1985).

RESULTS

The study participants were predominantly female, white, under the age of 40 years, well-educated and employed (Table 1). There was little difference between men and women except that men in the present study had higher levels of education ($P = 0{\cdot}02$), and women were somewhat more likely to be single, divorced, widowed or separated ($P = 0{\cdot}16$). Relatively few Seventh-day Adventists participated in the present study.

For the 100 participants the mean plasma GSH_t concentration was 761 μg/l with a standard deviation of 451 μg/l. Plasma GSH_t levels ranged from 86 to 2889 μg/l with a median value of 649 μg/l. For men the mean plasma GSH_t concentration was 924 μg/l, while for women the mean was 692 μg/l ($P = 0{\cdot}006$). Differences in plasma GSH_t level by race and by Seventh-day Adventist status could not be evaluated completely because all Seventh-day Adventists included in the present study were black, and because no black men participated in the present study who were not Seventh-day Adventists. Comparisons were made, therefore, among selected subgroups using the MSE based on all sample observations (Neter *et al.* 1985). Among men and women, Seventh-day Adventists had higher plasma GSH_t levels than did other race/religion subgroups. Among black women, Seventh-day Adventists were found to have much higher levels of plasma GSH_t than non-Seventh-day

Table 2. *Mean plasma total glutathione (GSH_t) concentration of the study participants, by race/Seventh-day Adventist (SDA) status and sex**

	Plasma GSH_t (μg/l)							
	Men				Women			
	n	Mean	Geometric mean	95% CI for geometric mean	*n*	Mean	Geometric mean	95% CI for geometric mean
Black SDA	3	1479	1301	736–2298	9	983	923	665–1282
Other Non-SDA†	4	1099	1092	667–1787	4	873	772	471–1263
White Non-SDA	23	822	745	606– 914	49	668	573	498– 660
Black Non-SDA	0	—	—	—	8	420	397	280– 562
Total	30	924	829	682–1006	70	692	594	524– 675

CI, confidence intervals.
* For details of procedures, see pp. 798–800.
† Includes Hispanic (one man) and Asian (three men, four women) races.

Table 3. *Mean plasma total glutathione (GSH_t) concentration by dietary status for black Seventh-day Adventists among the study participants**

	Plasma GSH_t (μg/l)			
	n	Mean	Adjusted† geometric mean	95% CI for adjusted† geometric mean
Vegetarian	6	1440	1388	(1127–1710)
Non-vegetarian	6	773	728	(591– 897)

CI, confidence intervals.
* For details of procedures, see pp. 798–800.
† Adjusted for sex.

Adventists ($P = 0{\cdot}003$; Table 2). White non-Seventh-day Adventist women tended to have higher concentrations of plasma GSH_t than did black non-Seventh-day Adventist women ($P = 0{\cdot}16$). Among whites only, plasma GSH_t levels were somewhat higher for men than for women ($P = 0{\cdot}12$).

Too few whites and non-Seventh-day Adventists consumed a vegetarian diet for a complete analysis of this factor among all study participants. However, among the twelve Seventh-day Adventists participating in the present study, half consumed a vegetarian diet. When adjusted for sex, Seventh-day Adventists on a vegetarian diet had higher levels of plasma GSH_t than Seventh-day Adventists who were not vegetarians ($P = 0{\cdot}002$; Table 3).

Because of the heterogeneity of relationships between plasma GSH_t concentration and race/Seventh-day Adventist status, and because of the small number of non-white men in the present study, further analyses were restricted to data collected on whites only.

The relationship between age and plasma GSH_t concentration appeared to be modified by sex. Plasma GSH_t levels increased with age in men but declined with advancing age in women (sex × age $P = 0{\cdot}05$; Table 4). There was essentially no variation in plasma GSH_t levels among groups defined by the demographic characteristics of marital status, education and employment status (values not shown).

Table 4. *Mean plasma total glutathione (GSH_t) concentration for white men and women among the study participants, by age and sex**

	Plasma GSH_t (μg/l)							
	Men				Women			
Age category (years)	*n*	Mean	Geometric mean	95% CI for geometric mean	*n*	Mean	Geometric mean	95% CI for geometric mean
19–29	8	630	596	417–852	15	862	721	555–936
30–39	7	881	783	534–1148	15	596	505	389–656
40–58	8	961	890	623–1273	19	571	529	420–668

CI, confidence intervals.
* For details of procedures, see pp. 798–800.

Table 5. *Mean plasma total glutathione (GSH_t) concentration among white study participants, by health conditions and health practices**

	Plasma GSH_t (μg/l)			
	n	Mean	Adjusted† geometric mean	95% CI for adjusted† geometric mean
Former cigarette smoker‡				
No	36	711	565	470–680
Yes	13	548	596	432–822
Currently taking oral contraceptives§				
No	18	871	747	580–961
Yes	3	462	417	225–773
Body mass index‖				
Normal	58	725	616	538–706
Overweight	14	681	654	496–864
Takes vitamins				
No	30	823	718	596–865
Yes: Irregular	21	558	501	401–625
Regularly	21	724	634	510–788
Consumes alcohol¶				
Never	8	610	604	420–869
Yes, Ever	64	730	626	552–709
Usual exercise				
More than once weekly	30	854	710	591–854
Once weekly	9	550	502	360–700
Less than once weekly	33	637	587	490–704

CI, confidence intervals.
* For details of participants and procedures, see pp. 798–800.
† Geometric means adjusted for sex and age in two categories (18–34 years and 35 years or older). For analyses restricted to a single sex, adjustment is for age only.
‡ Among women, since only one man was a former cigarette smoker.
§ Among women aged 18–34 years only. The geometric mean is unadjusted.
‖ (weight (kg))/(height (m))^2. Overweight = BMI ⩾ 27·8, men and BMI ⩾ 27·3, women.
¶ Includes consumption of beer, wine or spirits.

Table 6. *Mean plasma total glutathione (GSH_t) concentration among white study participants, by time interval between last meal and blood sampling and by time of day the blood sample was withdrawn**

	Plasma GSH_t (μg/l)			
	n	Mean	Adjusted† geometric mean	95% CI adjusted† geometric mean
Time interval between last meal and blood sampling				
0–1 h 59 min	13	638	590	444–783
2–3 h 59 min	34	695	584	489–696
4–5 h 59 min	15	796	708	542–924
6+ h	9	813	713	503–1010
Time of day of blood sampling (hours)				
09·30–11·29	31	666	578	485–690
11·30–13·29	17	748	691	544–876
13·30–15·29	11	889	822	609–1109
15·30–17·30	13	652	527	395–704

CI, confidence intervals.
* For details of participants and procedures, see pp. 798–800.
† Geometric means adjusted for sex and age in two categories (18–34 years and 35 years or older).

There was little variation in plasma GSH_t levels by other health characteristics and practices (Table 5). Among women, former use of cigarettes was not associated with plasma GSH_t level ($P = 0{\cdot}79$). In women under 35 years, oral contraceptive users appeared to have lower levels of plasma GSH_t ($P = 0{\cdot}10$). Body mass index ($P = 0{\cdot}80$) and usual level of exercise ($P = 0{\cdot}40$) were unrelated to plasma GSH_t concentration. Participants who consumed vitamins on an irregular basis had lower plasma GSH_t levels than non-users and regular vitamin consumers ($P = 0{\cdot}07$), but this affect did not appear to be consistent across all sex and age categories (category-specific data not shown). Alcohol consumers did not have reduced levels of plasma GSH_t ($P = 0{\cdot}54$). Other health-related factors which appeared unrelated to plasma GSH_t concentration included usual amount of sleep, family history of cancer, and current minor medical conditions such as asthma or hay fever (values not shown).

Two other factors which were evaluated in relation to plasma GSH_t levels were time-interval elapsed between last meal and blood sampling, and the time of day the blood sample was withdrawn (Table 6). Plasma GSH_t level varied little by either factor ($P > 0{\cdot}50$). Although plasma GSH_t concentration appeared to increase somewhat with increasing duration between last meal and blood sampling, confidence intervals about the sex- and age-adjusted geometric means overlapped widely. There was no apparent trend or difference in mean plasma GSH_t level with time of day the blood collection took place, although plasma GSH_t concentrations were somewhat higher in the early afternoon.

DISCUSSION

In the present exploratory study of 100 healthy volunteers we found a wide range of plasma GSH_t levels which appeared to vary by sex, age, and possibly by race. In addition, Seventh-day Adventists had higher levels of plasma GSH_t than did non-Seventh-day Adventists,

while among Seventh-day Adventists vegetarians appeared to have the highest concentrations of plasma GSH_t.

Glutathione may be important in disease prevention because of its role in preventing cellular injury and mutation by detoxifying xenobiotics (Stevens & Jones, 1989) and by protecting cells against oxidative damage, both directly as an antioxidant (Cerutti, 1985) and indirectly by maintaining other cellular antioxidants in a functional state (Wefers & Sies, 1988; Bendich, 1990). In addition, a number of studies indicate that glutathione may be involved in various aspects of the immune response, including T-cell and lymphocyte activation and proliferation (Hamilos & Wedner, 1985; Droge *et al.* 1986).

Although no prospective studies have examined the relationship between plasma glutathione status and disease risk, it has been shown that alcoholic subjects (Chawla *et al.* 1984; Lauterburg *et al.* 1984*b*), patients with malignancies (Beutler & Gelbart, 1985), HIV-seropositive individuals (Buhl *et al.* 1989) and those ingesting acetaminophen (Lauterburg *et al.* 1984*b*; Beutler & Gelbart, 1985) or diets deficient in ascorbic acid (Henning *et al.* 1991) have lower levels of plasma glutathione than do healthy subjects.

Plasma GSH_t levels were consistent with values reported in previous smaller studies of healthy humans. In those studies the mean values of plasma glutathione vary considerably, ranging from 104 to 5465 μg/l (Owens & Belcher, 1965; Hagenfeldt *et al.* 1978; Wendel & Cikryt, 1980; Chawla *et al.* 1984; Lauterburg *et al.* 1984*b*; Beutler & Gelbert, 1985; Burgunder & Lauterburg, 1986; Buhl *et al.* 1989; Hagen & Jones, 1989). This wide variation between studies may be due principally to differences in the forms of glutathione that were measured and in the laboratory methods used. In addition, because the concentration of glutathione in erythrocytes is much greater than in plasma (Beutler & Gelbart, 1985; Lash & Jones, 1985), small amounts of haemolysis can result in artifactually high plasma glutathione levels. For the present report all plasma samples with apparent haemolysis were excluded from the analyses.

In the present study men had much higher levels of plasma GSH_t than women. Studies of mice have demonstrated significantly higher levels of plasma glutathione in males than in females (Hirayama *et al.* 1987; Taniguchi *et al.* 1989), although this association has not been found in rats (Taniguchi *et al.* 1989). Two previous studies in humans, each with thirty participants, did not report a difference in plasma glutathione concentrations between men and women (Wendel & Cikryt, 1980; Beutler & Gelbart, 1985). Reasons for differences in glutathione levels by sex are not well understood, but research with animals indicates that plasma glutathione level may be affected by sex hormones. In mice the turnover rates of hepatic and renal glutathione appear to be significantly faster in males compared with females, with male castration decreasing these rates to levels approaching those of females. Several researchers have proposed therefore, that glutathione metabolism, secretion and transport may be influenced by sex hormones (Hirayama *et al.* 1987; Taniguchi *et al.* 1989). We found that, among young women, use of oestrogen-containing oral contraceptives was associated with lower plasma GSH_t levels, suggesting that the relationship between hormonal status, sex and plasma GSH_t concentration may be complex in humans. We could identify no other likely explanations for the observed difference in plasma GSH_t by sex. Men and women in the present study were similar in most characteristics except education and marital status, and plasma GSH_t concentration was not associated with those factors.

Among whites the association between age and plasma GSH_t concentration appeared to be modified by sex. Several studies of mice have demonstrated that tissue glutathione concentrations decline with age (Hazelton & Lang, 1980; Stohs *et al.* 1982), but sex-specific comparisons were not presented. The two previous studies in humans which examined sex differences in plasma glutathione concentration did not look at the combined effect of age

and sex (Wendel & Cikryt, 1980; Beutler & Gelbart, 1985), although one study reported no overall relationship between plasma glutathione level and age (Beutler & Gelbart, 1985). We know of no other nutritional biochemical measures that vary by age and sex in this manner and do not know why age might influence plasma GSH_t levels differently in men and women. This relationship should be considered in future studies of plasma glutathione in humans.

Plasma GSH_t concentration appeared to vary substantially by race and/or membership in the Seventh-day Adventist church, although we could not distinguish clearly between these factors. Diet may account partially for this relationship, as the Seventh-day Adventist religion endorses adherence to a lacto-ovovegetarian diet and other dietary restrictions (Phillips, 1975; Beeson *et al.* 1989). The Seventh-day Adventists participating in the present study who consumed vegetarian diets had much higher levels of plasma GSH_t than non-vegetarian Seventh-day Adventists. Although this comparison was based on small numbers of subjects in each group, the difference in mean plasma GSH_t concentration between these two groups was striking.

Diet may affect plasma glutathione level in a number of ways. In rodents, diets low in the glutathione-precursor amino acids methionine and cysteine have been shown to decrease hepatic glutathione concentration (Sendelbach *et al.* 1990) while a cysteine-rich diet increases liver glutathione levels (Bounous *et al.* 1989). Hepatic glutathione concentration has been found to decrease in riboflavin-deficient rats (Taniguchi *et al.* 1989). Although GSH is synthesized endogenously from its precursor amino acids (Meister, 1989), animal studies have shown that exogenous glutathione is transported into the cells of the small intestine as an intact tripeptide (Hagen & Jones, 1987). Orally administered glutathione increases plasma glutathione levels in rats (Hagen *et al.* 1990; Aw *et al.* 1991) and in humans (Hagen & Jones, 1989).

Dietary glutathione is available from a variety of food sources. Meats contain the highest concentrations, with large amounts in fruits and vegetables and little or none in grains and dairy products (Jones *et al.* 1992). Thus, differences in diet may explain some differences in plasma levels. However, because of the relatively high concentrations of glutathione and its precursor amino acids in meats (Jones *et al.* 1992), it is uncertain that vegetarian Seventh-day Adventists consume more glutathione precursor amino acids or more intact glutathione. To our knowledge, no studies in humans have yet examined the relationship between diet and plasma glutathione concentration.

Other more complex dietary or health-related factors may affect plasma glutathione levels. In the present study participants who took vitamins irregularly appeared to have lower levels of plasma GSH_t than those not taking any vitamins or taking vitamins on a regular basis. This effect, however, was not consistent across all sex and age categories examined, and may be spurious or due to other lifestyle factors associated with irregular vitamin usage. Although a recent study of healthy men receiving ascorbic acid-deficient diets demonstrated decreased plasma glutathione levels (Henning *et al.* 1991), the relationships between the plasma antioxidants ascorbic acid, α-tocopherol, β-carotene and glutathione appear to be quite complex (Wefers & Sies, 1988; Frei *et al.* 1989; Bendich, 1990) and it is unclear whether ingestion of vitamins, particularly antioxidants, should theoretically increase or decrease plasma GSH_t levels.

Among white participants plasma GSH_t concentration did not vary significantly by either time-interval between last meal and blood sampling, or time of day that the blood sample was taken. However, a slight increasing trend in plasma GSH_t level was observed with increasing duration between the most recent meal and blood sampling. In rats fasting appears to increase hepatic glutathione synthesis (Lauterburg & Mitchell, 1981) with a concomitant increase in efflux of the reduced (GSH) and oxidized (GSSG) forms of glutathione (Lauterburg *et al.* 1984*a*), and it is possible that a similar phenomenon exists

in humans. Although diurnal variations in rat blood glutathione levels have been observed, these fluctuations appear to be limited to erythrocyte glutathione (Calcutt, 1967).

Several limitations to the present study should be noted. Although the study is, to our knowledge, the largest conducted to date which examines plasma glutathione concentrations in free-living humans, the numbers of subjects in the analysis subgroups become small with increasing number of simultaneously examined factors. This reduces the statistical power of subgroup comparisons, and for this reason we chose to adjust only for sex and age, the two most important factors associated with plasma GSH_t level.

An additional limitation is that most participants in the present study were recruited from among employees and students in health-related organizations. Other study subjects were members of a Seventh-day Adventist congregation. Both groups of participants are likely to differ from members of the general population on a number of health-related factors. This, along with the self-referred nature of the study participants, precludes generalization of these findings to the population at large.

It is also important to note that in the present study we assayed plasma GSH_t, which consists of GSH and all disulphide forms, including mixed disulphides with cysteine and protein. Many previous investigations of plasma glutathione assessed a combination of GSH and GSSG forms of glutathione, but did not assay for the presence of plasma glutathione in the form of mixed disulphides. In rats, however, approximately 70% of plasma glutathione exists in the protein-bound form (Lash & Jones, 1985), and recent studies in both rats and humans have shown that, like glutathione, protein-bound glutathione appears to increase in response to orally administered glutathione (Hagen & Jones, 1989; Aw *et al.* 1991). Protein-bound glutathione may participate in regulating the biological activity of plasma peptide hormones and immunoglobulins and receptor binding to plasma membranes (Lash & Jones, 1985). Because of this, plasma GSH_t, which includes the potentially biologically important disulphide forms, was assayed in the present study.

In conclusion, from the present study it is apparent that plasma GSH_t concentrations vary widely in humans and are influenced by sex and age. Because research indicates a vegetarian diet is associated with reduced risk of some chronic diseases (Dwyer, 1988; National Research Council Committee on Diet and Health, 1989) and because Seventh-day Adventists are also at reduced risk of certain chronic diseases (Phillips, 1975; Phillips *et al.*, 1978), it was particularly intriguing that plasma GSH_t levels were higher among Seventh-day Adventists and among vegetarians. Because glutathione has several biological functions that may influence risk of chronic diseases, further studies are needed to examine factors affecting plasma GSH_t status, particularly dietary practices, and the relationship between plasma GSH_t and subsequent disease incidence.

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