

GLUTATHIONE IN HUMAN PLASMA: DECLINE IN ASSOCIATION WITH AGING, AGE-RELATED MACULAR DEGENERATION, AND DIABETES

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Abstract—Blood samples were analyzed for GSH and GSH redox state in 40 age-related macular degeneration (ARMD) patients (> 60 y), 33 non-ARMD diabetic patients (> 60 years), 27 similarly aged non-ARMD and nondiabetic individuals (> 60 years), and 19 younger individuals (< 60 years) without ARMD or diabetes. Results showed a significantly lower plasma GSH in older individuals (ARMD, diabetes, and controls) than in younger individuals ($p < .01$). Total GSH (GSH_t) obtained following treatment with dithiothreitol was significantly lower only in diabetic cases ($p < .05$) but also approached significance for ARMD cases ($p = .089$). Estimation of redox potential indicated that the plasma GSH pool is considerably more oxidized in all of the older groups. Analyses of whole blood GSH showed that GSH was significantly lower in diabetic cases compared to the other groups, but did not reveal any difference associated with age or ARMD. In contrast, GSSG in whole blood was significantly higher in the older groups compared to the younger controls. The results suggest that in studies of age-related pathologies, oxidation of GSH may be a more important parameter than a decline in pool size, while in specific pathologies such as diabetes, both oxidation and a decline in pool size may be important. © 1998 Elsevier Science Inc.

Keywords—Glutathione, Glutathione disulfide, Redox state, Blood plasma, Human, Macular degeneration, Diabetes, Aging, Antioxidants, Free radical

INTRODUCTION

Glutathione is an important antioxidant that functions directly in elimination of toxic peroxides and aldehydes and indirectly in maintaining vitamins C and E in their reduced and functional forms.^{1–3} Vitamin C deficiency results in decreased plasma GSH⁴ and vitamin E supplementation increases plasma GSH.⁵ Both vitamin C and E concentrations in plasma decrease with age,^{6,7} suggesting that GSH may also decrease in plasma with age. A study of total GSH (GSH_t; GSH plus oxidized forms) in plasma of individuals aged 18–61 also suggested that GSH may decline with age⁸ and Kretzschmar and Muller⁹ reported that the mean plasma GSH for five individuals age 18–35 years was higher than five individuals age 36–45, and seven individuals age 46–57.

Lang et al.¹⁰ found decreased erythrocyte GSH in association with aging. Because of the important antioxidant roles of GSH, such declines in plasma or tissue GSH could contribute to development and/or progression of age-related toxicities and diseases.

Alternatively, several disease processes have been associated with decreased plasma GSH. For instance, alcoholic cirrhosis results in decreased plasma GSH, apparently due to decreased hepatic GSH synthesis and release.¹¹ Infection with human immunodeficiency virus is associated with decreased plasma GSH^{12,13} due to a systemic decrease in synthesis.¹⁴ Decreased plasma GSH has also been found in patients with cystic fibrosis,¹⁵ and animal studies show that protein malnutrition and chronic hypoxia also result in decreased plasma GSH.¹⁶ Thus, progression of a chronic disease may also cause a decrease in plasma GSH.

The purpose of the present study was to determine whether plasma GSH is decreased in patients with reti-

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nopathies associated with two common age-related diseases, ARMD, and diabetes, and to see whether changes are unique to the disease or associated with aging. ARMD is thought to occur as a consequence of oxidative injury to the retinal pigment epithelium.^{17,18} Increased plasma levels of lipid peroxides¹⁷ and decreased plasma antioxidants¹⁹ have been found in subjects with ARMD. Type II diabetes is a common age-related disease with several associated complications, including diabetic retinopathy, which could be associated with oxidative processes. Previous studies have shown that the erythrocyte GSH is decreased^{20–22} and plasma GSSG is increased in diabetes.²³ In addition, plasma lipid peroxide concentrations are increased.²⁴ The present results show that diabetic patients and possibly ARMD patients have a smaller and more oxidized plasma GSH pool than similarly aged, unaffected individuals. In addition, the results suggest that an oxidation of the plasma GSH pool may be an important change associated with aging even if the GSH pool size does not change markedly.

MATERIALS AND METHODS

Recruitment and study population

This study was reviewed and approved by the Investigational Review Board of Emory University and performed in accordance with the ethical standards in the 1975 Declaration of Helsinki, as revised in 1983. Participants gave their informed consent prior to inclusion in the study. Older subjects (> 60 years) were recruited from patients of the Retina Service at the Emory Eye Clinic and individuals who accompanied them. Patients carrying the diagnosis of ARMD had a minimum of five drusen in each eye, with some vision loss related to the macular disease. Diabetic patients were diagnosed through chart history; the majority were type II diabetics managed for diabetic retinopathy. Younger subjects (< 60) were recruited from Emory University employees and students. Blood samples were collected during June–August 1992. Although some blacks were included in the data collection, blacks were excluded from analyses because there were no black ARMD cases and a previous study showed that plasma GSH differs between whites and nonwhites.⁸ Because we had no way to control for smoking and smoking is known to affect antioxidant status, data for smokers were also excluded from analysis.

Data collection

Subjects were interviewed to obtain data on race/ethnic background, age, education, and vitamin and mineral supplement intake. Blood was obtained by venipunc-

ture and processed immediately so that plasma was added to ice-cold 5-sulfosalicylic acid at 2 min after blood was drawn. In preliminary studies using blood collection into evacuated tubes (Vacutainers), some samples had a slight reddish color suggesting that hemolysis can occur by this procedure. We therefore compared GSH_t values for plasma obtained following blood collection into Vacutainers with that obtained from blood of the same individuals collected with a butterfly needle and syringe. The results showed that mean values for the Vacutainer method (7.86 ± 0.66) were higher than values for plasma obtained by the butterfly needle/syringe method even though the samples were processed and analyzed identically after the blood draw. We concluded that the higher values obtained with the Vacutainer method were probably due to slight hemolysis (which was visible in some samples and verified spectrophotometrically²⁵). Thus, to avoid erroneously high values for GSH due to hemolysis, blood was collected using a butterfly needle and syringe.

Blood (0.8 ml) was immediately dripped from the syringe into Eppendorf tubes containing 50 mM acivicin (7.5 μ l), 0.2% (w/v) heparin (100 μ l), and 16.2 mM bathophenanthroline disulfonic acid (92.5 μ l). Tubes were inverted to mix and spun in a microcentrifuge for 30 s. Plasma (0.5 ml) was transferred to ice-cold 10% (w/v) 5-sulfosalicylic acid (0.25 ml) at 2 min after the blood was withdrawn into the syringe. This standardized processing minimized interindividual variation due to GSH loss during collection. Our previous studies indicate that there is approximately 20% loss of GSH during the 2-min processing time,²⁶ and no efforts were made to correct for this loss. However, samples were treated identically so that all should be affected similarly. Acivicin was included to protect against GSH loss due to degradation by γ -glutamyltranspeptidase. Comparison of GSH values obtained with acivicin to values for plasma collected without acivicin or with another inhibitor, serine-borate, indicated that degradation by this enzyme during the 2-min collection and processing time does not affect the results. Samples were stored at -70°C for up to 2 weeks prior to derivatization and analyzed within 2 weeks of derivatization. Stability studies showed no significant loss of GSH for this duration of storage. For whole-blood analyses, 0.5 ml blood was added to ice-cold 10% (w/v) 5-sulfosalicylic acid (1 ml) and stored at -70°C .

Plasma GSH_t samples were neutralized and treated with 10 mM dithiothreitol for 15 min,²⁶ a condition that gave maximal GSH concentrations. Each sample was then acidified with 0.5 volumes of 30% (w/v) trichloroacetic acid and spun for 2 min in a microcentrifuge. Samples were extracted with methanol:chloroform (2:1) to remove lipids and derivatized at pH 8 with 40 mM

Table 1. Demographic Characteristics Excluding Smokers and Blacks

	% Male	Median Age (Range)	% Using Vitamin Supplements	Median Years Education (Range)
Controls < 60y (<i>n</i> = 19)	42.1 (<i>n</i> = 8)	31 (19 – 42)	N/A	N/A
Controls > 60y (<i>n</i> = 27)	44.4 (<i>n</i> = 12)	72 (62 – 87)	48.1 (<i>n</i> = 13)	12 (5 – 17)
ARMD cases (<i>n</i> = 40)	40.0 (<i>n</i> = 16)	74 (63 – 93)	75.0 (<i>n</i> = 30)	13 (7 – 17)
Diabetes cases (<i>n</i> = 33)	45.4 (<i>n</i> = 15)	71 (60 – 80)	63.6 (<i>n</i> = 21)	13 (4 – 20)

iodoacetic acid and 1-fluoro-2,4-dinitrobenzene [1.5% (v/v) in absolute ethanol] as described by Fariss and Reed²⁷ except that the derivatization time was increased to > 8 h. GSH and GSSG derivatives were separated by high performance liquid chromatography (HPLC) on a gradient HPLC with an Ultrasil amine column (Beckman) and quantified relative to standards by absorbance at 365 nm.²⁷ Signal was linear down to 0.5 μ M GSH with an injection volume of 100 μ l.

Dithiothreitol was purchased from Baxter Diagnostics Incorporated, McGaw Park, IL. Methanol was HPLC grade (Fisher Scientific, Pittsburgh, PA). All other chemicals were purchased from Sigma Chemical Company, St. Louis, MO.

Statistics

Analysis of variance (ANOVA) was used to compare blood levels among cases and controls. Statistical analyses were done on log-transformed data and geometric means and SEM are reported. Because plasma GSHT differs for males and females,⁸ analyses reported were controlled for sex. Without controlling for sex, results were comparable for all analyses except comparison of GSHT in plasma of ARMD vs. > 60 years controls. In this case, a significant difference ($p < .05$) was present without controlling for sex but was lost when data were controlled for sex ($p = .089$).

RESULTS

Table 1 shows the basic demographic characteristics of participants. Cases and older controls had similar age, sex, and educational distributions. However, a greater proportion of ARMD cases than diabetic cases or controls were taking vitamin supplements.

A comparison of GSH concentrations (Fig. 1) showed that younger individuals had significantly higher plasma GSH than the older controls or patients ($p < .01$). The diabetic patients had lower GSH values than the older controls ($p < .02$), but values for ARMD patients were not significantly lower than older controls. The results for GSHT (Fig. 1) showed that the diabetic cases had significantly lower plasma GSHT ($p < .05$) than the older controls and that there was a trend toward lower GSHT in

the ARMD subjects compared to older controls ($p = .089$). However, there was no significant difference in the GSHT between the older and younger controls. Thus, the results suggest that the decline in GSH with aging is largely due to a decrease in GSH, per se, while diabetes and ARMD are associated with a decrease in the total GSH pool (GSHT). This could occur if there is an age-related decline in the ratio of reduced to oxidized forms of GSH.

When the amount of glutathionyl moiety present as oxidized forms is calculated as the difference between GSHT and GSH in plasma (Fig. 1), the results show that the older groups have substantially more glutathione present as disulfides in the blood plasma. If one assumes that all of the oxidized moiety is present as GSSG, one can estimate the redox potential (E_h) of the GSH/GSSG redox couple in the plasma from the Nernst equation and these mean values (Table 2). The results suggest that the E_h is about 45 mV more oxidized in the older subjects. A difference (19 to 24 mV) is also apparent between the values for diabetics and the values for ARMD and older

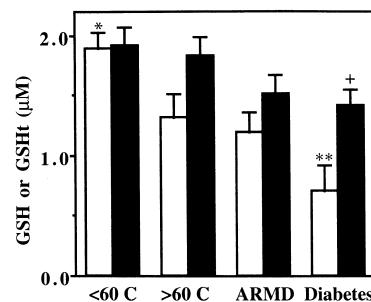


Fig. 1. Plasma GSH and GSHT levels in individuals under 60 years and in individuals over 60 years without and with ARMD or diabetes. GSH is given as open bars. Data shown are the geometric mean (\pm SEM) for < 60 years controls ($n = 19$), > 60 years controls ($n = 27$), ARMD patients ($n = 40$), and diabetic patients ($n = 33$). Geometric means (and 95% confidence intervals) for younger, older, ARMD and diabetic subjects are (in μ M): 1.89 (1.66, 2.14), 1.32 (1.09, 1.60), 1.18 (1.00, 1.41), and 0.71 (0.51, 0.98), respectively. *Significantly different from older groups, $p < .01$. **Significantly different from both control groups, $p < .02$. GSHT is given as filled bars and includes reduced glutathione, glutathione disulfide, and other disulfide forms obtained following treatment with dithiothreitol. Data shown are the geometric means of plasma GSHT \pm SEM. Geometric means (and 95% confidence intervals) for younger controls, older controls, ARMD subjects, and diabetic subjects are (in μ M): 1.92 (1.65, 2.25), 1.86 (1.56, 2.14), 1.48 (1.28, 1.78), and 1.41 (1.16, 1.72), respectively. +Significantly different from older and younger controls ($p < .05$).

Table 2. Calculated Redox Potential (E_h) for GSH Pool in Blood Plasma and Erythrocytes of Younger and Older Controls and Patients with ARMD or Diabetes^a

Group	Plasma GSSG (μM)		Plasma GSSG (μM)		
	Estimated as (GSH _t -GSH)/2	Plasma E_h^b (mV)	Estimated as (GSH _t -GSH)/4	Plasma E_h^c (mV)	Erythrocyte E_h (mV)
Controls < 60 Gy	0.015	-131	0.008	-140	-193
Controls > 60 Gy	0.27	-84	0.135	-93	-192
ARMD	0.15	-89	0.075	-98	-188
Diabetes	0.35	-65	0.175	-74	-179

^a Values were calculated as $E_h = E_o + RT/2F \ln [GSSG]/[GSH]^2$ where E_o was taken as -0.24V^{32} using data given in Fig. 1.

^b GSSG was estimated as $(GSH_t - GSH)/2$.

^c Values were calculated with $GSSG = (GSH_t - GSH)/4$, based upon direct measurements of GSSG and the mixed disulfide of GSH and cysteine in human plasma which show that about half of the total oxidized GSH is present as GSSG.²⁸

controls. E_h values are shifted 9 mV more reducing if one assumes that half of the oxidized GSH is present as GSSG and the other half as a mixed disulfide with cysteine²⁸ (Table 2), but the pattern of differences remain. Thus, the results indicate that a decline in antioxidant status of the GSH pool may occur independently of the change in pool size described above.

Because previous studies have shown that decreased erythrocyte GSH is associated with aging¹⁰ and diabetes,²⁰⁻²² we examined whether a shift in redox state of the erythrocyte GSH pool was also present. For this, we used whole-blood measurements because erythrocytes account for almost all of the whole-blood GSH. The results show that GSH is significantly lower in the diabetic group, but the ARMD cases, older controls and younger controls were not significantly different from each other (Fig. 2). In contrast, the whole-blood GSSG was significantly lower in the younger individuals ($p < .05$) compared to either older group (Fig. 2). The ARMD, diabetic, and older controls were not significantly different from each other.

Calculations of E_h for the different conditions (Table 2) show that the GSH/GSSG pool is more oxidized in the erythrocytes of diabetics than either of the control groups or the ARMD group. Of interest, the E_h is the same in the older and younger control groups, indicating that the redox difference observed in the plasma is not present in the erythrocytes. Thus, the significantly lower GSSG in the erythrocytes of the younger controls (see above) may be a result of differences in pool size rather than being a consequence of a difference in redox status. The values for the ARMD patients were not sufficiently different (4 to 5 mV) from the controls to know whether this represents a real difference from the controls and will require additional investigation.

DISCUSSION

The activity of GSH as an antioxidant can be expressed in two ways: as a function of GSH concentration,

and as a function of the redox state of the GSH/GSSG pool. Enzymes that utilize GSH as a substrate may be dependent solely upon the concentration of GSH while redox-sensitive processes may be dependent upon the redox state of GSH. At present, it is not clear which of these parameters is more important as a potential risk factor for age-related toxicities or an indicator of oxidative stress. The present results suggest that an oxidation of the plasma GSH pool may be an important change associated with aging even if the GSH pool size does not change markedly. In contrast, in diabetes and probably ARMD, a change in pool size occurs in addition to a change in redox state.

The analytical procedure used for GSH measurement is an HPLC method with absorbance detection that has

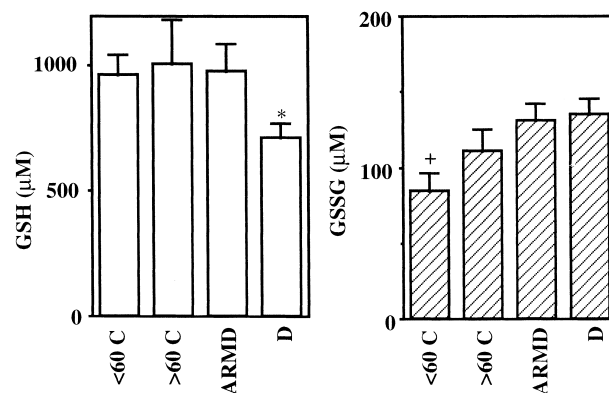


Fig. 2. Whole-blood GSH and GSSG levels in individuals under 60 years and in individuals over 60 years without and with ARMD or diabetes. Data shown by open bars (left-hand scale) are the geometric means of whole blood GSH for young individuals ($n = 19$), older controls ($n = 27$), ARMD patients ($n = 40$), and diabetic patients ($n = 33$). Geometric means (and 95% confidence intervals) for young, older, ARMD and diabetic subjects are (in μM): 962 (885, 1004), 1006 (842, 1202), 976 (870, 1093), and 713 (609, 835), respectively. *Significantly different from other groups, $p < .05$. Data shown by hatched bars are the geometric means of whole-blood GSSG \pm SEM. Geometric means (and 95% confidence intervals) for young, older, ARMD, and diabetic subjects are (in μM): 84 (64, 111), 111 (84, 147), 131 (110, 157), and 135 (117, 156), respectively. +Significantly different from ARMD and diabetic patients, $p < .01$.

been used widely for measurement of GSH and GSSG. The method is sufficiently sensitive to measure GSH in human plasma but does not allow quantitation of GSSG or other oxidized forms. Consequently, the approach used measured GSH directly and the total GSH (GSH_t) following reduction with dithiothreitol. Subsequent measurement of oxidized forms of GSH in plasma of 10 healthy individuals (20–40 years) with a more sensitive fluorometric method has shown that about half of the oxidized GSH is present as GSSG and half as a disulfide with cysteine.²⁸ Thus, the lack of a significant difference between GSH and GSH_t in the younger age group in the present study appears to be due to variability in the assay rather than due to the absence of GSH-containing disulfides in the plasma of the younger individuals.

Animal studies have shown that liver and plasma GSH undergo diurnal variations as a consequence of eating patterns;²⁹ studies of human bone marrow have also revealed a diurnal variation.³⁰ In a previous study of plasma GSH_t in humans, we found no effect of time interval between the most recent meal and the plasma value.⁸ However, in a study of diurnal variation in 17 young, healthy subjects, we found that values were relatively constant during the day (0800–1700 h) except for transient increases within an hour of eating.³¹ Although we did not control the time of sampling, the time between the last meal and blood sampling was at least 1 h for most, if not all, of the subjects. Thus, diurnal variations or differences in eating habits between the groups appears to be an unlikely explanation for the observed differences in GSH and GSH redox state.

Calculation of E_h required use of an estimate of the standard potential E_o for the 2 GSH/GSSG couple. For this purpose, the value of -240 mV³² was used. As other estimates of E_o are in the range of -230 to -260 for pH 7.0^{33–34} and a pH effect of about 30 mV/pH unit is expected,³³ the calculated E_h cannot be considered a precise measure of E_h . However, the conclusions made concerning the differences in E_h between groups are likely to reflect real differences in E_h because calculations assuming that only half of the oxidized GSH is present as GSSG resulted in the same characteristics as when it was assumed that all was present as GSSG (see Table 2).

An oxidation of the thiol-disulfide redox state may have dramatic effects on cell functions because transcriptional regulation of gene expression is sensitive to redox state³⁵ and changes in redox affect cell proliferation rate³⁶ and apoptosis.³⁷ Consequently, the apparent shift in the plasma GSH pool to a more oxidized state may be an important factor in the risk of many age-related toxicities and pathologies. The current study shows that the E_h for diabetic cases is more positive (oxidized) than for the other groups, which may reflect a further disease-

related oxidative stress. The E_h for ARMD is not different from that of the similarly aged controls, but the ARMD patients reported higher vitamin supplement intake than the controls. Thus, for ARMD, higher intake of antioxidant vitamins, which has been started after onset of the disease, could prevent detection of a difference in the redox state that contributed to disease development.

Previous studies have shown that serum antioxidants such as Vitamin E decrease with advancing age.⁷ A decrease in vitamins C, E, and β -carotene in serum has also been found in subjects with ARMD,²⁰ and decreases in vitamin C and β -carotene were found in experimentally induced diabetes in rats.³⁸ Thus, the observed differences in GSH concentration and redox status in the plasma are consistent with an emerging view that antioxidant defenses decline with aging and age-related disease processes.

The mechanistic basis for differences in GSH and GSH redox state in association with aging and/or diabetes or macular degeneration is not clear. Ookhtens and Mittur³⁹ found that plasma GSH in mature rats is mostly cleared by conversion to cysteine or cystine. Assuming that this data is relevant to humans, an increase in γ -glutamyltranspeptidase activity could account for a decreased GSH_t due to increased degradation. Alternatively, a decrease in GSH could occur due to decreased release from tissues, either as a consequence of decreased intracellular concentrations or due to decreased GSH transporter functions with aging. An oxidation of GSH in plasma could also reflect a generalized oxidative stress or a decline in antioxidant systems in tissues. GSSG appears to be released from most cells as a consequence of oxidative stress so that an oxidation of the cellular pool could shift the balance of GSH and GSSG efflux and change the extracellular redox state.

In contrast to the plasma values, the E_h for the GSH pool in erythrocytes was not altered in association with age. However, the data suggest that the GSH/GSSG redox state is more oxidized in diabetic cases, and possibly ARMD cases. Previous studies have shown a decreased level of erythrocyte GSH and an increase in GSSG in diabetes.^{20–23} Such changes could occur in part because of decreased synthesis of GSH; decreased activity of γ -glutamylcysteine synthetase has been previously found in erythrocytes of diabetics.²¹ Because decreased synthesis cannot readily explain the observed difference in E_h , it appears likely that other effects, such as increased use of GSH to detoxify peroxides or decreased activity of glucose 6-phosphate dehydrogenase or GSSG reductase, contribute to the differences observed.²³

In summary, the estimated plasma GSH pool in individuals > 60 years was substantially oxidized when compared to individuals < 60 years. The plasma GSH pool in diabetic and possibly ARMD cases were

more oxidized than similarly aged controls. The erythrocyte pools were similarly oxidized in diabetic and possibly ARMD cases; however, no age-related decrease in redox status of the erythrocyte was found. The results are consistent with the interpretation that there is a generalized oxidation associated with aging that may contribute to the development of age-related toxicities and pathology.

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REFERENCES

1. Reed, D. J. Glutathione: Toxicologic implications. *Annu. Rev. Pharmacol. Toxicol.* **30**:603–631; 1990.
2. Wefers, H.; Sies, H. Antioxidant effects of ascorbate and glutathione in microsomal lipid peroxidation are dependent on vitamin E. In: Poli, G.; Cheeseman, K. H.; Dianzani, M. U.; Slater, T. F., eds. *Advances in biosciences*, vol. 76: *Free radicals in the pathogenesis of liver injury*. New York: Pergamon Press; 1989:309–316.
3. Winkler, B. S.; Orselli, S. M.; Rex, T. S. The redox couple between glutathione and ascorbic acid: A chemical and physiological perspective. *Free Radic. Biol. Med.* **17**:333–349; 1994.
4. Henning, S. M.; Zhang, J. Z.; McKee, R. W.; Swenseid, M. E.; Jacob, R. A. Glutathione blood levels and other oxidants defense indices in men fed diets low in vitamin C. *J. Nutr.* **121**:1969–1975; 1991.
5. Costagliola, C.; Menzione, M. Effect of vitamin E on the oxidative state of glutathione in plasma. *Clin. Physiol. Biochem.* **8**:140–143; 1990.
6. Rikans, L. E.; Moore, D. R. Effect of aging on aqueous-phase antioxidants in tissues of male Fischer rats. *Biochim. Biophys. Acta* **966**:269–275; 1988.
7. Vandewoude M. F. J.; Vandewoude, M. G. Vitamin E status in normal population: The influence of age. *J. Am. Coll. Nutr.* **6**:307–311; 1987.
8. Flagg, E. W.; Coates, R. J.; Jones, D. P.; Eley, J. W.; Gunter, E. W.; Jackson, B.; Greenberg, R. S. Plasma total glutathione in humans and its association with demographic and health-related factors. *Br. J. Nutr.* **70**:797–808; 1993.
9. Kretzschmar, M.; Müller, D. Aging, training and exercise. A review of effects on plasma glutathione and lipid peroxides. *Sports Med.* **10**:196–209, 1993.
10. Lang, C. A.; Naryshkin, S.; Schneider, D. L.; Mills, B. J.; Lindeman, R. D. Low blood glutathione levels in healthy aging adults. *J. Lab. Clin. Med.* **120**:720–725; 1992.
11. Chawla, R. K.; Lewis, F. W.; Kutner, M. H.; Bate, D. M.; Roy, R. G. B.; Rudman, D. Plasma cysteine, cystine, and glutathione in cirrhosis. *Gastroenterology* **87**:770–776; 1984.
12. Buhl, R.; Holroyd, K. J.; Mastrangeli, A.; Cantin, A. M.; Jaffe, H. A.; Wells, F. B.; Saltini, C.; Crystal, R. G. Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. *Lancet* **ii**:1294–1298; 1989.
13. Eck, H.-P.; Gmunder, H.; Hartman, M.; Petzoldt, D.; Daniel, V.; Droge, W.; Low concentration of acid-soluble thiol (cysteine) in the blood plasma of HIV-1 infected patients. *Biol. Chem. Hoppe-Seyler* **370**:101–108; 1989.
14. Smith, C. V.; Jones, D. P.; Guenther, T. M.; Lash, L. H.; Lauterburg, B. H. Compartmentation of glutathione. Implications for the study of toxicity and disease. *Toxicol. Appl. Pharmacol.* **140**:1–12; 1996.
15. Roum, J. H.; Buhl, R.; McElvaney, N. G.; Borok, Z.; Crystal, R. G. Systemic deficiency of glutathione in cystic fibrosis. *J. Appl. Physiol.* **75**:2419–2424; 1993.
16. Bai, C. Jones, D. P. GSH transport and GSH-dependent detoxication in small intestine of rats of exposed in vivo to hypoxia. *Am. J. Physiol.* **271**:G701–G706; 1996.
17. Young, R. W. Pathophysiology of age-related macular degeneration. *Surv. Ophthalmol.* **31**:291–306; 1987.
18. Young, R. W. Solar radiation and age-related macular degeneration. *Surv. Ophthalmol.* **32**:252–269; 1988.
19. Eye Disease Case Control Study Group. Antioxidant status and neovascular age-related macular degeneration. *Arch. Ophthalmol.* **111**:104–109; 1993.
20. Bono, A.; Caimi, G.; Catania, A.; Sarno, A.; Pandolfo, L. Red cell peroxide metabolism in diabetes mellitus. *Horm. Metab. Res.* **19**:264–266; 1987.
21. Murakami, K.; Kondo, T.; Ohtsuku Y.; Fujiwara, Y.; Shimada, M.; Kawakami, Y. Impairment of glutathione metabolism in erythrocytes from patients with diabetes mellitus. *Metabolism* **38**:753–758; 1989.
22. Forrester, T. E.; Badaloo, V.; Bennett, F. I.; Jackson, A. A. Excessive excretion of 5-oxoproline and decreased levels of blood glutathione in type II diabetes mellitus. *Eur. J. Clin. Nutr.* **44**:847–850; 1990.
23. Costagliola, C.; Iuliano, G.; Menzione, M.; Nesti, A.; Simonelli, F.; Rinaldi, E. Systemic human diseases as oxidative risk factors in cataractogenesis. I. Diabetes. *Ophthalm. Res.* **20**:308–316; 1988.
24. Kaji, H.; Kurasaki, K.; Ito, K.; Saito, T.; Saito, K.; Nijoke, T.; Kojima, Y.; Ohsaki, Y.; Ide, H.; Tsuji, M. Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type II (non-insulin dependent) diabetic women. *Klin. Wochenschr.* **63**:765–768; 1985.
25. Oser, B. L. *Hawk's physiological chemistry*, 14th ed. New York: McGraw Hill; 1965: 1096.
26. Lash, L. H.; Jones, D. P. Distribution of oxidized and reduced forms of glutathione and cysteine in rat plasma. *Arch. Biochem. Biophys.* **240**:583–592; 1985.
27. Fariss, M. W.; Reed, D. J. High-performance liquid chromatography of thiols and disulfides: Dinitrophenol derivatives. *Methods Enzymol.* **143**:101–109; 1987.
28. Jones, D. P.; Kurtz, J. C.; Sternberg, P. Thiol and disulfide forms of glutathione (GSH) cysteine (Cys), cysteinylglycine (CG) and homocysteine (HC) in human plasma. *FASEB J.* **11**:A150, 1997.
29. Jaeschke, H.; Wendel, A. Diurnal fluctuation and pharmacological alteration of mouse organ glutathione content. *Biochem. Pharmacol.* **34**:1029–1033; 1985.
30. Smaaland, R.; Svardal, A. M.; Lote, K.; Ueland, P. M.; Laerum, O. D. Glutathione content in human bone marrow and circadian stage relation to DNA synthesis. *J. Natl. Cancer Inst.* **83**:1092–1098; 1991.
31. Samiec, P. Regulation and utilization of extracellular GSH. Ph.D. Dissertation. Emory University, Atlanta, GA; 1995.
32. Rost, J.; Rapoport, S. Reduction potential of glutathione. *Nature* **201**:185; 1964.
33. Clark, W. M. *Oxidation-reduction potentials of organic systems*. Baltimore: Williams & Wilkins; 1960.
34. Gilbert, H. F. Molecular and cellular aspects of thiol-disulfide exchange. *Adv. Enzymol. Related Areas Mol. Biol.* **63**: 69–172; 1990.
35. Abate, C.; Patel, L.; Rauscher, F. J.; Curran, T. Redox regulation of fos and jun DNA-binding activity in vitro. *Science* **249**:1157–1161; 1990.
36. Hwang, C.; Sinskey, A. J. The role of oxidation-reduction potential in monitoring growth of cultured mammalian cells. In: Spier, R. E.; Griffiths, J. B.; Meignier, B.; eds. *Production of biologicals from animal cells in culture*. Oxford: Halley Court; 1991:548–567.
37. Malorni, W.; Rivabene, R.; Santini, M. T.; Donelli, G. N-Acetylcysteine inhibits apoptosis and decreases viral particles in HIV-chronically infected U937 cells. *FEBS Lett.* **327**:75–78; 1993.
38. Young, I. S.; Torney, J. J.; Trimble, E. R. The effect of ascorbate supplementation on oxidative stress in the streptozotocin diabetic rat. *Free Radic. Biol. Med.* **13**:41–46; 1992.
39. Ookhtens, M.; Mittur, A. V. Developmental changes in plasma thiol-disulfide turnover in rats: A multicompartmental approach. *Am. J. Physiol.* **267**:R415–R425; 1994.