Blood glutathione concentrations in a large-scale human study

JOHN P. RICHIE, JR.,* LISA SKOWRONSKI, PATRICIA ABRAHAM, and YVONNE LEUTZINGER

Little is known about the variability of blood glutathione (GSH) in human subjects. Thus, we wanted to develop and validate a rapid method for measuring GSH concentrations in whole blood and apply this method to ascertain the variation of GSH in a large-scale study of free-living adults. The assay was highly sensitive (detection limit <5 pmol) and precise, with an interassay variation of 2.3% and a sampling variation of 3.6%. Applying this method to screen 715 adults, we observed a threefold range of GSH concentrations, with a mean of 1.02 mmol/L and CV of 17%. GSH concentrations were 8–10% greater in smokers than in nonsmokers (P <0.001). Although we observed no sex differences for GSH, GSH/hemoglobin ratios were 8–18% greater in women <55 years old than in older subjects (P <0.05). The results presented here validate the use of this method for large-scale human studies and provide information on the variation and normal values of blood GSH in adults.

INDEXING TERMS: screening • reference values • antioxidants • enzymatic methods

Glutathione (GSH), a ubiquitous cellular antioxidant, plays a central role in defense against a variety of diseases and both exogenous and endogenous insults.1 Its functions include the detoxification of xenobiotics, carcinogens, free radicals, and peroxides; regulation of immune function; and maintenance of protein structure, function, and turnover [1, 2]. Low concentrations of GSH have been implicated in numerous pathological conditions, including diabetes [3], alcoholic liver disease [4], AIDS [5, 6], acute hemorrhagic gastric erosions [7], cataracts [8], Parkinson disease [9], xenobiotic-induced oxidative stress and toxicity [10], and aging [11–13].

Given these essential roles of GSH in cellular protection and homeostasis, GSH availability may be a key factor in the maintenance of health, and GSH concentrations may serve as a useful indicator of disease risk in humans [12]. Investigating these relationships will require large-scale epidemiological and clinical studies in which GSH status is measured.

Previous results suggest that blood GSH may serve as an accurate indicator of GSH status in human subjects. GSH is present as an intracellular component in blood at concentrations of ~1 mmol/L, with the amounts in plasma representing <1% of the total. In laboratory animals, blood concentrations of GSH reflect the concentrations in other, less-accessible tissues such as liver and kidney [11]. In the mouse, for example, the decreases in blood GSH seen during aging are highly correlated with similar decreases in other tissues [14]. Conversely, blood GSH concentrations are not affected by conditions that result in transient changes in the GSH content of specific tissues, such as fasting or circadian fluctuations [15] or acetaminophen administration [14]. Although fewer such studies have been performed in human subjects, an aging-related decrease in blood GSH has been reported in the elderly [12, 16, 17]. Furthermore, recent data suggest that those elderly with low GSH concentrations are in poorer health than those with higher GSH [13].

Although these results support the use of blood GSH as an indicator of the GSH status of an individual, to date, little information is available on the variability of blood concentrations of GSH in human populations, and methods to obtain such information have not been described. Thus, our objectives were to develop and validate a rapid and specific method for measuring whole-blood GSH in large-scale human studies and to apply this method to determine the variability of blood GSH concentrations in the population.

Materials and Methods

GENERAL PROCEDURES

Blood collection. Venous blood was collected from the antecubital vein into Vacutainer Tubes (Becton Dickinson, Franklin Lakes, NJ) containing heparin or EDTA as an anticoagulant. Capillary blood was drawn by finger puncture with a sterile lancet (Becton Dickinson) and collected into 300-μL capillary vials (Sarstedt, Princeton, NJ) containing lithium heparin as an anticoagulant. For the comparison of GSH concentrations in venous vs capillary blood samples, a total of 16 subjects (9 women and 7
men) were recruited from laboratory personnel. After they provided informed consent according to the AHF Internal Review Board requirements, capillary and venous blood samples were obtained as described above. The time between sampling was <10 min for each subject. For the determination of intraindividual variation in blood GSH concentrations, four subjects were recruited as described above; each subject supplied 5–10 sequential capillary blood samples, collected from different fingers.

**Sample preparation.** Optimal sample processing procedures were based on previous studies [18, 19]. Immediately after collection, samples were deproteinized by addition of four volumes of ice-cold 50 g/L metaphosphoric acid (MPA). After 10 to 20 min, acid extracts were obtained by centrifugation at 13 000g for 2 min.

**Reagents and calibrators.** The assay buffer consisted of 100 mmol/L NaH₂PO₄ and 5 mmol/L EDTA adjusted to pH 7.5 with NaOH. Solutions of 1.26 mmol/L, 5.5′-dithiobis-(2-nitrobenzoic acid) (DTNB), 0.72 mmol/L NADPH, and 2.5 kU/L glutathione oxidoreductase (EC 1.6.4.2, type IV; Sigma Chemical Co., St. Louis, MO) were prepared in assay buffer on the day of use. The DTNB solution was maintained at room temperature and the NADPH and glutathione reductase solutions were kept at 0–4°C.

Calibrators were prepared as follows: A stock solution of 3.26 mmol/L GSH in distilled H₂O was aliquoted into cryovials and stored at −20 °C. This solution was stable for at least 1 year. On the day of the assay, an aliquot was thawed at room temperature and eight working calibrators ranging from 0 to 19.5 μmol/L were prepared by diluting in 2 g/L MPA, the same MPA concentration as the processed samples. Glutathione disulfide (GSSG) calibrators were also prepared and gave equivalent results.

**PROcedures**

**Assays.** Just before analysis, the MPA extracts were diluted 20-fold in assay buffer. Then 50 μL of each working calibrator or diluted sample extract was added to each well of a flat-bottomed 96-well microtiter plate (Immulon 1; Dynatech Labs., Chantilly, VA). Both calibrators and samples were analyzed in duplicate in adjacent columns of wells. Plates were configured so that the calibrators were in the first two columns and 40 samples in duplicate were in the remaining columns. Using an eight-channel pipettor, we then added 50 μL each of DTNB and glutathione oxidoreductase solution to each well. After incubating this for >5 min at room temperature, we started the reaction by adding 50 μL of NADPH solution to each well, again using a multichannel pipettor. Within 30–120 s after addition of the NADPH, we transferred the plate to a plate reader (MR700; Dynatech Labs.), which monitored at 2-min intervals for 6 min the rate of color change at 410 nm. Both GSH and GSSG were measured simultaneously by this method. Because GSSG represents only a small percentage of total acid-soluble (free) glutathione, results were presented as GSH + GSSG (tGSH) and expressed in units of GSH equivalents.

For comparison, GSH and GSSG were also measured in some samples by HPLC with dual electrochemical detection [20]. To determine the hemoglobin (Hb) content, an aliquot of blood was hemolyzed in distilled H₂O and analyzed spectrophotometrically, using Drabkin’s reagent [21].

**Quality control.** To maintain assay precision and accuracy over long periods, we performed several quality-control measures: External calibrators were included on every assay plate, and the range of concentrations in the calibration curve encompassed the range of expected sample GSH values. All samples were run in duplicate and, in some cases, at different dilutions to determine reproducibility and proportionality of assay responses. Recovery experiments were performed by adding 10 mmol/L GSH or GSSG to five samples of freshly obtained whole blood to give final concentrations of 0, 0.005, 0.05, and 1.0 mmol/L and processing the blood as described above.

An important aspect of quality control was the inclusion of control samples on each assay plate. These controls were prepared from pooled blood that had been processed with MPA as described above. The MPA extract was aliquoted into cryovials and stored at −20 °C. Before freezing, aliquots (>20) were removed and assayed for tGSH to obtain a reference interval for control values (mean ± SD). An aliquot was thawed on each day of analysis and included on every assay plate. Control values, all of which fell within the established control range, were tracked over time to monitor assay performance and precision. The control samples were stable for at least 1 year.

**Population study**

To determine the feasibility of this method in large-scale human studies and establish a database of interindividual variation in GSH concentrations in free-living adults, we conducted a cross-sectional screening study of blood GSH concentrations. As part of a cholesterol screening, we recruited 715 subjects (484 men and 231 women, ages 20 to 70 years) from attendees of the annual meeting of the National Grocers Association in San Francisco (February 1993). Characteristics of the study subjects are summarized in Table 1. After obtaining informed consent from each subject, we collected fingerstick blood samples and processed them on-site with MPA. Acid extracts were frozen and shipped on solid CO₂, stored at −20 °C, and later analyzed for tGSH and Hb as described above. In addition to blood samples, questionnaire data on age, sex, and smoking status were obtained from each individual.

**Statistical analysis**

Data are expressed as means ± SD. Differences between groups were considered significant at P <0.05 by Student’s t-test or by ANOVA with Scheffé’s posthoc test.

**Results**

**Assay validation**

Titrations of DTNB and NADPH to determine optimal assay concentrations for routine use led us to select final assay concentrations of 0.32 mmol/L DTNB and 0.18 mmol/L NADPH. Initial rates were also dependent on the concentra-
tions of GSSG reductase, as were the background rates in reaction mixtures with no sample added. A final enzyme activity of 0.125 U/well provided sufficient sensitivity with minimal assay background (<0.005 A/min). Using these optimized conditions, we assessed intraassay variability with samples processed and analyzed simultaneously in replicates of 25–45. CVs ranged from 1.0% to 3.2% (mean 2.3%). Analytical recovery of GSH and GSSG added to blood at concentrations of 0.005–1.0 mmol/L was between 99% and 102%. The minimal detectable limits (noise x 3) were 5 pmol for GSH and 2.5 pmol for GSSG, representing 0.1 μmol/L GSH and 0.05 μmol/L GSSG in blood. GSH calibration curves were linear from 15 to 1200 pmol/well and were independent of MPA concentrations of <20 g/L. A slight reduction in the assay rate occurs when MPA concentrations exceed 20 g/L.

The stability of tGSH in human blood was assessed at various temperatures (Fig. 1). When blood was frozen immediately at −70 °C, GSH concentrations were stable for at least 3 weeks. However, at −20 °C and 4 °C, substantial losses of tGSH occurred within days, indicating that processing should be done immediately after the collection of blood. Once processed, tGSH concentrations were stable in MPA extracts for >1 year at −20 °C, 7 days at 0 °C, and 1 day at room temperature (22 °C); at 37 °C, almost 25% of GSH was lost within 24 h. In addition, repeated freeze-thawing of processed samples in MPA did not substantially affect tGSH measurements. To try to simplify sample-processing procedures, we also assessed GSH stability in samples stored at −20 °C after addition of MPA but before centrifugation and in samples to which solid MPA had been added. However, in both procedures, recovery of added GSH was incomplete.

A comparison between the tGSH content of capillary and venous blood yielded the same results (GSH meq/L) for both sample types: venous (0.987 ± 0.253) and capillary (0.987 ± 0.306); moreover, these values were highly correlated (r = 0.92).

Finally, in selected samples, tGSH concentrations were also determined by HPLC with electrochemical detection; equivalent results were obtained (Table 2).

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**Table 1. Characteristics of study subjects.**

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;34</td>
<td>62 (12.8)</td>
<td>49 (21.2)</td>
<td>111 (15.5)</td>
</tr>
<tr>
<td>34–44</td>
<td>159 (32.9)</td>
<td>67 (29.0)</td>
<td>226 (31.6)</td>
</tr>
<tr>
<td>45–54</td>
<td>155 (32.0)</td>
<td>73 (31.6)</td>
<td>228 (31.9)</td>
</tr>
<tr>
<td>55–64</td>
<td>84 (17.4)</td>
<td>33 (14.3)</td>
<td>117 (16.4)</td>
</tr>
<tr>
<td>≥65</td>
<td>24 (5.0)</td>
<td>9 (3.9)</td>
<td>33 (4.6)</td>
</tr>
</tbody>
</table>

**Smoking status**

| Nonsmokers | 428 (89.4) | 203 (89.0) | 631 (89.2) |
| Smokers    | 51 (10.6)  | 25 (11.0)  | 76 (10.7)  |
| CPD ≤15    | 21 (41.2)  | 13 (52.0)  | 34 (44.7)  |
| CPD >15    | 30 (58.8)  | 12 (48.0)  | 42 (55.3)  |

CPD, current no. of cigarettes smoked per day.

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Fig. 1. Stability of glutathione in human blood at various temperatures. Aliquots of freshly obtained blood were either processed with MPA immediately for GSH analysis, or stored at 4 °C (○), −20 °C (□), or −70 °C (■). After 4–23 days, samples were thawed, if necessary, and processed with MPA for tGSH analysis. Symbols and bars represent mean and SD values, respectively; bars are omitted if SD values are smaller than the size of the symbol.

Sampling variation was determined by obtaining repeat (5–10) capillary blood samples from four individuals and analyzing for tGSH. The CVs ranged from 2.7% to 4.8% with a mean of 3.6% (Table 3).

**Table 2. Two methods compared for determining glutathione (meq/L) in human blood.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Micromethod tGSH</th>
<th>HPLC-DEC tGSH</th>
<th>GSH</th>
<th>GSSG</th>
<th>tGSH</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.02</td>
<td>0.948</td>
<td>0.052</td>
<td>1.00</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.13</td>
<td>1.08</td>
<td>0.087</td>
<td>1.17</td>
<td>0.966</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.850</td>
<td>0.822</td>
<td>0.053</td>
<td>0.875</td>
<td>0.971</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.04</td>
<td>1.02</td>
<td>0.083</td>
<td>1.10</td>
<td>0.945</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.46</td>
<td>1.40</td>
<td>0.048</td>
<td>1.45</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.921</td>
<td>0.872</td>
<td>0.038</td>
<td>0.910</td>
<td>1.01</td>
<td></td>
</tr>
</tbody>
</table>

Mean: 0.987

* Micromethod [tGSH]/HPLC-DEC [tGSH].

DEC, electrochemical detection.

**Table 3. Sampling variation in blood GSH concentrations.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>Mean ± SD tGSH, meq/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>1.01 ± 0.037</td>
<td>3.7</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.817 ± 0.039</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.904 ± 0.029</td>
<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1.20 ± 0.032</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Mean: 3.6
Table 4. Blood hemoglobin and cholesterol concentrations in 715 subjects, by age and sex.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Hb, g/L*</th>
<th>Cholesterol, mmol/L*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>160 ± 36.9</td>
<td>4.84 ± 1.17</td>
</tr>
<tr>
<td>35–44</td>
<td>158 ± 24.4</td>
<td>5.31 ± 0.981</td>
</tr>
<tr>
<td>45–54</td>
<td>157 ± 23.8</td>
<td>5.65 ± 1.11</td>
</tr>
<tr>
<td>55–64</td>
<td>156 ± 29.2</td>
<td>5.61 ± 0.97g c d</td>
</tr>
<tr>
<td>&gt;64</td>
<td>162 ± 33.3</td>
<td>5.52 ± 0.805</td>
</tr>
</tbody>
</table>

* Values are mean ± SD. No. of subjects in each group is given in Table 1.

Significantly different from men (P <0.05).

Significantly different from <35-year age group (P =0.05).

Significantly different from 35–44-year age group (P =0.05).

Significantly different from 45–54-year age group (P <0.05).

Significantly different from <35-, 35–44-, and 45–54-year age groups (P <0.05).

Table 5. Blood glutathione concentrations: mean ± SD (range).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>meq/L</th>
<th>CV, %</th>
<th>tGSH/Hb</th>
<th>μeq/g</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>484</td>
<td>1.02 ± 0.167 (0.67-1.60)</td>
<td>16.4</td>
<td>6.68 ± 1.47 (2.83-11.7)</td>
<td>18.1</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>231</td>
<td>1.03 ± 0.187 (0.67-1.90)</td>
<td></td>
<td>7.39 ± 1.87 (3.09-14.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

observed for the men, but increases of 13–16% were observed in women <64 years (P <0.05). Plasma cholesterol concentrations were 5–8% greater in men than in women for ages <55 years and 10–14% lower in men for ages >55 years (P <0.05). Progressive increases with age were observed in both sexes, similar to previous reports [22, 23].

A mean ± SD tGSH for all subjects was 1.02 ± 0.173 meq/L, similar to that obtained in previous, albeit smaller, reported studies (Table 5) [24, 25]. A nearly threefold range for tGSH and a fivefold range for tGSH/Hb were observed for all subjects (Fig. 2), resulting in CVs of 17% for tGSH and 23% for tGSH/Hb. No age-related differences were observed for individuals <55 years old (Table 6). In contrast, albeit the number of individuals older than 65 years was small (13 men and 8 women), the women in this age group had a 10–20% decrease in tGSH/Hb values. In general, tGSH/Hb values were 8–18% greater in women than in men for all age groups younger than 55 years but not in older groups, and no gender differences were observed for tGSH. Smokers had higher tGSH concentrations than nonsmokers, but the differences were small: 8–10% (Table 7). The increase in tGSH was similar for both light (<15 cigarettes per day) and heavy (>15 cigarettes per day) smokers. There were no correlations between tGSH and other measurements, e.g., serum cholesterol, number of cigarettes smoked, or state of residence.

Discussion

Numerous laboratory studies have suggested that GSH is a critical factor in protecting organisms against toxicity and disease. Blood GSH concentrations may serve as an indicator of GSH status and, thus, disease risk in human subjects. However, little information is available on the stability and variation of blood GSH within individuals and its variation in the population. Few studies have analyzed whole blood or erythrocyte GSH in healthy subjects. Lang et al. [12] examined the blood GSH concentrations in 170 subjects and found a significant increase (P <0.001) in the proportion of elderly individuals with

![Fig. 2. Frequency distribution of blood GSH concentrations.](image-url)
low GSH values compared with younger adults. Similar findings have been observed in two smaller studies [16, 17]. Recently, a relationship between blood GSH concentrations and overall health status in a sample of elderly in Michigan was observed [13]. These results support the use of blood GSH as an indicator of health status, particularly during aging. They also point to the need for large-scale human studies to further elucidate the relationship of blood GSH with health status and disease risk.

To this end, we developed and validated a method for GSH that allows for the measurement of several hundred samples per day. The assay is based on the DTNB–enzymatic recycling method of Teitz [25] and Owens and Belcher [26]. A similar method was independently developed for use with animal tissues [27]; in the present report we demonstrate the validity and applicability of this procedure for use in human blood samples and in large-scale human studies.

We placed particular emphasis on the validation of this method, which we based on several factors, including reproducibility, accuracy, recovery, and sensitivity. Assay and sampling variation, 2.3% and 3.6%, respectively, were minimal, and recovery of known quantities added to actual samples (99–102%) was excellent. The assay was sensitive, with minimal detectable quantities as low as 5 pmol of GSH, equivalent to a concentration of 0.1 μmol/L GSH in blood or tissues. Stringent quality assurance measures were developed and included the routine analysis of control reference samples, sample replicates, and assay variation and recovery.

Optimal conditions for sample processing and storage were determined by expanding upon previous results [18, 19]. GSH was stable in freshly drawn blood for at least 4 h, allowing for blood collection to be feasible in clinical situations. The use of blood samples obtained by fingerstick was verified as being equivalent to venous blood. Processing, which consists of a single-step extraction with MPA followed by centrifugation, can be accomplished with minimal equipment in a clinical laboratory, in a doctor’s office, or in the field. Finally, MPA extracts are stable at −20°C for at least 1 year, which allows for convenient storage and shipping for large long-term studies.

To examine the interindividual variation of blood GSH, we performed a large-scale screening study. Glutathione concentration in 715 subjects exhibited a threefold range with a mean of 1.02 meq/L and an interindividual CV of 17%. We also calculated GSH content per gram of Hb to account for possible variations in hematological variables, given that GSH is primarily an erythrocytic component. Expressed in this fashion, GSH concentrations exhibited a fivefold range with a mean of 7.17 μeq/g Hb and an interindividual CV of 23%. The observed variation was not related to sex differences or to age in individuals <65 years old.

Smokers tended to have higher GSH concentrations than nonsmokers. However, the difference was small (10%) and the percentage of smokers was low (10%), so that smoking status accounted for very little of the overall variation. A similar increase in blood GSH in smokers has been observed in smaller studies [28, 29]. One explanation is that increased GSH concentrations are an adaptive response to the chronic oxidative challenge presented by cigarette smoking. Indeed, decreases in the antioxidant/nutrients vitamin C and β-carotene have been observed previously in smokers [30].

Previous studies had found lower GSH in older individuals [12, 16, 17], but in the present study the number of elderly subjects was small. A decrease in GSH/Hb during aging was apparent only in the sample of women >65 years old but apparently resulted from an increase in Hb content rather than a loss of GSH. The sex differences in GSH/Hb noted for subjects <55 years old were also due to lower values for Hb in premenopausal women than in age-matched men, and no sex differences were observed in GSH concentrations.

Although numerous prospective, retrospective, and cohort studies have been performed examining the relationships between antioxidant/nutrients such as β-carotene, vitamin A, α-tocopherol, and ascorbic acid and disease risk [31–33], similar studies with GSH, the major endogenous antioxidant, have not been performed. The present study was not designed to examine these relationships, because personal health histories and physical examinations were not available; however, our data clearly

### Table 6. Blood glutathione concentrations by age and sex (mean ± SD).

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Men</th>
<th>Women</th>
<th>tGSH/Hb, μeq/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>1.04 ± 0.15</td>
<td>1.02 ± 0.19</td>
<td>6.84 ± 1.50</td>
</tr>
<tr>
<td>35–44</td>
<td>1.01 ± 0.17</td>
<td>1.05 ± 0.16</td>
<td>6.55 ± 1.53</td>
</tr>
<tr>
<td>45–54</td>
<td>1.00 ± 0.16</td>
<td>1.04 ± 0.18</td>
<td>6.55 ± 1.34</td>
</tr>
<tr>
<td>55–64</td>
<td>1.04 ± 0.18</td>
<td>1.02 ± 0.25</td>
<td>6.90 ± 1.50</td>
</tr>
<tr>
<td>&gt;64</td>
<td>1.05 ± 0.20</td>
<td>0.95 ± 0.11</td>
<td>6.74 ± 1.66</td>
</tr>
</tbody>
</table>

* Significantly different from men (P <0.05).

### Table 7. Effect of smoking on blood glutathione concentrations.

<table>
<thead>
<tr>
<th>Group</th>
<th>tGSH, meq/L</th>
<th>tGSH/Hb, μeq/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td>1.01 ± 0.171</td>
<td>6.81 ± 1.59</td>
</tr>
<tr>
<td>Smokers</td>
<td>1.10 ± 0.173*</td>
<td>7.23 ± 1.58*</td>
</tr>
<tr>
<td>≤15 CPD</td>
<td>1.11 ± 0.207*</td>
<td>7.21 ± 1.84*</td>
</tr>
<tr>
<td>&gt;15 CPD</td>
<td>1.10 ± 0.151*</td>
<td>7.25 ± 1.37*</td>
</tr>
</tbody>
</table>

* Significantly different from nonsmoker group (P <0.001).

CPD, current no. of cigarettes smoked per day.
demonstrate the feasibility and underline the importance of such investigations. Large-scale metabolic epidemiological studies providing detailed health information and relating it to GSH status are needed to establish the basis for using GSH status as an indicator of disease and (or) aging. Further, the relationship of GSH status to diet and other environmental and lifestyle factors needs to be examined.

Most of the previous studies examining the association of GSH status with disease or nutrition in human subjects relied on the measurement of plasma GSH [34, 35]. Plasma GSH has been reported to reflect liver GSH concentrations and export in rodents [36], but such may not be the case in humans [37]; other studies with rats suggest that erythrocytes may be substantially more important in this regard [38]. In addition, the measurement of GSH in plasma can present problems, given the very low concentrations and instability of GSH in plasma. Indeed, without the addition of stabilizing compounds such as DTNB to blood immediately after collection, GSH contents in plasma are rapidly depleted [39, 40]. In a recent study of 100 participants, a very wide (30-fold) range of plasma GSH was found, with values apparently not correlating with any health measures [41]. Finally, few data are available on the stability and variability of plasma GSH within and between individuals.

Overall, the results of the assay presented here demonstrate its validity for measuring blood GSH concentrations in large-scale human studies. This method can be successfully applied in future studies aimed at elucidating the role of GSH in human health and disease.

We thank Joshua Muscat for his assistance with data analysis, Ellen Louis for her help in sample processing, and Marie-Ange Brunemann for her editorial assistance. This study was supported in part by NIH grant DE09514.

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