Free Radical Biology and Medicine 65 (2013) 742–749

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contributions

The concentration of glutathione in human erythrocytes is a heritable trait

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ARTICLE INFO

Article history:
Received 3 June 2013
Received in revised form
31 July 2013
Accepted 2 August 2013
Available online 9 August 2013

Keywords:
Glutathione
Red blood cells
Heritability
Twin study
Free radicals

ABSTRACT

Glutathione (GSH) is a ubiquitous, redox-active, small molecule that is critical to cellular and organism health. In red blood cells (RBCs), the influence of the environment (e.g., diet and lifestyle) on GSH levels has been demonstrated in numerous studies. However, it remains unknown if levels of GSH are determined principally by environmental factors or if there is a genetic component, i.e., heritability. To investigate this we conducted a twin study. Twin studies are performed by comparing the similarity in phenotypes between mono- and dizygotic twin pairs. We determined the heritability of GSH, as well as its oxidation product glutathione disulfide (GSSG), the sum of GSH equivalents (tGSH), and the status of the GSSG/2GSH couple (marker of oxidation status, E_{hc}) in RBCs. In our study population we found that the estimated heritability for the intracellular concentration of GSH in RBCs was 57 %; for GSSG it was 51 %, tGSH 63 %, and E_{hc} 70 %. We conclude that a major portion of the phenotype of these traits is controlled genetically. We anticipate that these heritabilities will also be reflected in other cell types. The discovery that genetics plays a major role in the innate levels of redox-active species in RBCs is paradigm shifting and opens new avenues of research in the field of redox biology. Inherited RBC antioxidant levels may be important disease modifiers. By identifying the relative contributions of genes and the environment to antioxidant variation between individuals, new therapeutic strategies can be developed. Understanding the genetic determinants of these inherited traits may allow personalized approaches to relevant therapies.

Glutathione (GSH)[1] is an important redox-active biomolecule critical in the maintenance and regulation of cellular and organismal health. Significantly lower population-mean levels of GSH in red blood cells (RBCs) have been indicated in many disease states, including but not limited to acute exposure to drugs and toxins [1–3], protein malnutrition [4], hormonal imbalance [5], genitourinary disease [6], gastrointestinal disease [6], cancer [6], cardiovascular disease [6], musculoskeletal disease [6], Parkinson disease [7], adult respiratory distress syndrome [8], diabetes mellitus [9,10], liver disease [11], AIDS [12], cataracts [13], and aging [14,15]. On the other hand, high availability of GSH in RBCs has been correlated with longevity in mosquitos [16] and mice [17] and good health in elderly humans [18].

Major hurdles remain before levels of GSH can be used as a therapeutic target or as a diagnostic biomarker. Central questions remaining to be fully answered are: (a) does disease alter the levels of GSH in RBCs; (b) does the level of GSH influence the risk for disease of a population; and (c) what other factors determine the cellular and tissue levels of GSH. The goal of this research is to determine if there is a heritable component to observed levels of GSH in human RBCs.

Multiple studies of healthy individuals have observed a wide interindividual range of GSH levels in RBCs (0.4 to 3.0 mM, Table 1). This large interindividual range (coefficient of variation (CV) ≈ 36 % for the data in Table 1) is present regardless of age or method of detection. In contrast, intraindividual (i.e., within an individual) levels of GSH in RBCs are relatively stable over time (< 10 % CV over a period of several months, 15 % over several

Abbreviations: BDD, boron-doped diamond electrode; DZ, dizygotic; GSH, glutathione; GSSG, glutathione disulfide; ICC, intraclass correlation coefficient; MZ, monozygotic; RBC, red blood cell; tGSH, total GSH equivalents, i.e., [tGSH]=[GSH]+2[GSSG].

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http://dx.doi.org/10.1016/j.freeradbiomed.2013.08.002

Table 1
Observed ranges of glutathione in human RBCs.

GSH[a]				GSH/mM[b]			Population	Method[c]	Ref.
Mean	SD	Range	Units	Mean	SD	Range			
70	16.2	37–107	mg/100 cc pRBCs[d]	1.8[e]	0.4	0.9–2.6	Healthy adults ($n=117$)	I	[47]
71	16.6	31–118	mg/100 cc pRBCs	1.8[e]	0.4	0.8–2.9	Non-Ashkenazi subjects	I	[48]
73	11.9	42–114	mg/100 cc pRBCs	1.9[e]	0.3	1.0–2.8	Ashkenazi subjects	I	[48]
76	6.5	62–85	mg/100 cc pRBCs	2.0[e]	0.2	1.5–2.1	G6PD normal patients ($n=25$)	I	[49]
34	8.3	23–70	mg/100 cc pRBCs	0.9[e]	0.2	0.6–1.7	Sensitive Iranian males ($n=35$)	I	[50]
64	12.3	40–112	mg/100 cc pRBCs	1.6[e]	0.3	1.0–2.7	Nonsensitive Iranian males ($n=257$)	I	[50]
44	11.0	27–60	mg/100 cc pRBCs	1.1[e]	0.3	0.7–1.5	Sensitive Iranian females ($n=12$)	I	[50]
67	15.6	36–124	mg/100 cc pRBCs	1.7[e]	0.3	0.9–3.0	Nonsensitive Iranian females ($n=132$)	I	[50]
63	—	46–81	mg/100 cc pRBCs	1.6[e]	—	1.1–2.0	Random samples ($n=15$)	II	[51]
64	—	49–85	mg/100 cc pRBCs	1.6[e]	—	1.2–2.1	Random samples ($n=15$)	III	[51]
68	10.2	47–99	mg/100 cc pRBCs	1.7[e]	0.3	1.1–2.4	Normal blood donors ($n=85$)	III	[51]
80	12.9	52–118	mg/100 cc pRBCs	2.1[e]	0.3	1.3–2.9	Normal infants ($n=253$)	III	[51]
486	85	343–728	μmol/L whole blood	1.2[f]	0.2	0.8–1.8	Healthy adults ($n=47$)	IV	[52]
337	138	—	μmol/L whole blood	0.8[f]	0.3	—	Elderly subjects ($n=64$)	IV	[52]
3.5	1.8	—	μM/g Hgb	1.2[g]	0.6	—	Young (31 ± 10 years, $n=33$)	I	[53]
2.3	0.9	—	μM/g Hgb	0.8[g]	0.3	—	Aged (69 ± 11 years, $n=28$)	I	[53]
849	63	400–1400	μmol/L RBCs	0.8	0.6	0.4–1.4	Healthy adults (18–73 years, 107 men, 94 women)	V	[54]
1.0	0.2	0.7–1.6	meq/L RBCs	1.0	0.2	0.7–1.6	Healthy men ($n=484$)	VI	[55]
1.0	0.2	0.7–1.9	meq/L RBCs	1.0	0.2	0.7–1.9	Healthy women ($n=231$)	VI	[55]
85	24.4	54–110	mg/100 cc pRBCs	2.2[e]	0.6	1.4–2.8	Healthy adults ($n=30$)	I	[56]
1549	133	—	pmol/10^7cells	1.7[h]	0.1	—	Unknown population	VII	[57]
1.0	0.1	0.6–1.4	mmol/L RBCs	1.0	0.1	0.6–1.4	Healthy adults ($n=10$)	VI	[19]
1133	63.5	1000–1220	μmol/L RBCs	1.1	0.1	1.0–1.2	Healthy individuals ages 0.2–1 year ($n=25$)	VI	[58]
1235	131	1038–1500	μmol/L RBCs	1.2	0.1	1.0–1.5	Healthy individuals ages 2–11 years ($n=28$)	VI	[58]
1257	99	1071–1473	μmol/L RBCs	1.3	0.1	1.1–1.5	Healthy individuals ages 12–24 years ($n=23$)	VI	[58]
1226	83	1118–1408	μmol/L RBCs	1.2	0.1	1.1–1.4	Healthy individuals ages 25–40 years ($n=40$)	VI	[58]
1024	137	745–1300	μmol/L RBCs	1.0	0.1	0.7–1.3	Healthy individuals ages 41–69 years ($n=60$)	VI	[58]
Overall mean, SD, and range				**1.4**[i]	**0.5**	**0.4–3.0**			

[a] Values as reported in the units provided.
[b] Reported values converted to an estimated intracellular concentration (mM).
[c] Methods used to measure GSH: I, Beutler et al. [59]; II, chlorpromazine displacement; III, nitroprusside; IV, capillary electrophoresis; V, HPLC with postcolumn derivatization and fluorimetric detection; VI, DTNB; VII, isotope dilution liquid chromatography–tandem mass spectrometry.
[d] pRBCs, packed red blood cells.
[e] Assuming packed RBC hematocrit is 80 %.
[f] Assuming an average hematocrit of 40 %.
[g] Assuming average hemoglobin (Hgb) of 140 g/L in whole blood and a hematocrit of 40%.
[h] Assuming a mean corpuscular volume of 90 fL.
[i] Mean is not weighted.

years) [19,20]. This suggests that GSH levels are maintained at an innate level that is distinct between individuals; leading to the hypothesis that a major portion of the variability observed in GSH and glutathione disulfide (GSSG) concentrations and half-cell reduction potential (E_{hc}) status in RBCs is determined by genotypic differences.

To test this hypothesis we performed a classic twin study. Such a twin study compares the similarity of a trait in monozygotic (MZ) and dizygotic (DZ) twins [21,22]. For twin studies, this measure of similarity has been the intraclass correlation (ICC) [23]. With the ICC values for MZ and DZ twins for a given trait, the proportion of the observable interindividual variation attributed to genotypic differences (i.e., heritability) can be estimated [24].

Materials and methods

Institutional Review Board approval

The study was approved by the Human Subjects Office of The University of Iowa. Subjects were qualified for participation by meeting criteria for autologous blood donation according to standard operating procedures of The University of Iowa DeGowin Blood Center. Standard health history and demographic information were obtained at the time of enrollment and informed consent.

Recruitment

Twins (19 pairs; 14 MZ and 5 DZ pairs) were recruited to donate blood at the DeGowin Blood Center at the University of Iowa Hospitals and Clinics. Before standard blood donation, samples of whole blood for this study were collected. Twins were not required to come in as a pair, nor were there restrictions on the time of day for donations. This resulted in individuals within pairs donating weeks apart and at different times of the day. Information on ethnicity or smoking status of subjects enrolled in the trial is not available; these topics are not queried on the standard health history form used to determine eligibility for donation of whole blood.

Zygosity testing

The zygosity of the twin pairs was determined by isolating DNA from white blood cells (WBCs). White blood cells were obtained from leuko-reduction filters, utilized during the processing of the whole blood donation into components. Dulbecco's phosphate-buffered saline (DPBS; 15 mL) was pushed through the filter to extract the WBCs. The DPBS with WBCs was collected into a 50-mL centrifuge tube. The 50-mL tube was centrifuged at 500 *g* for 10 min. WBCs were resuspended in 2 mL of DPBS. DNA was extracted using the AutoGen (Holliston, MA, USA) QuickGene-610 L nucleic acid extraction machine with the Fuji QuickGene DNA whole blood kit (AutoGen), following manufacturer's instructions. Genotype was