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Phlorhizin Protects against Erythrocyte Cell Membrane Scrambling

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ABSTRACT: Phlorhizin interferes with glucose transport. Glucose depletion triggers suicidal erythrocyte death or eryptosis, characterized by cell shrinkage and cell membrane scrambling. Eryptosis is further triggered by oxidative stress. The present study explored whether phlorhizin influences eryptosis following glucose depletion or oxidative stress. Cell membrane scrambling was estimated from annexin binding, cell volume from forward scatter (FSC), and cytosolic Ca^{2+} concentration from Fluo-3 fluorescence. Phlorhizin (10−100 μM) added alone did not modify scrambling, FSC, or Fluo-3 fluorescence. Glucose depletion (48 h) significantly increased Fluo-3 fluorescence, decreased FSC, and increased annexin binding, effects in part significantly blunted by phlorhizin (annexin binding ≥ 10 μM, FSC ≥ 50 μM). Oxidative stress (30 min 0.3 mM *tert*-butylhydroperoxide) again significantly increased Fluo-3 fluorescence and triggered annexin binding, effects again in part significantly blunted by phlorhizin (Fluo-3 fluorescence ≥ 50 μM, annexin-binding ≥ 10 μM). Phlorhizin did not blunt the cell shrinkage induced by oxidative stress. The present observations disclose a novel effect of phlorhizin, that is, an influence on suicidal erythrocyte death following energy depletion and oxidative stress.

KEYWORDS: phosphatidylserine, scrambling, calcium, cell volume, eryptosis

INTRODUCTION

The naturally occurring phenolic compound phlorhizin (phloretin 2′-*O*-glucoside, phlorizin, phlorrhizin, phloridzin, or phlorizoside)[1−7] is a potent inhibitor of the Na^+-coupled glucose transporter SGLT1.[8−13] Inhibition of SGLT1 could impair intestinal glucose absorption as well as part of renal glucose reabsorption and thus counteract obesity, diabetes, and diabetic nephropathy.[13,14] On the other hand, SGLT1 inhibition may foster pathogen-induced apoptosis of intestinal cells[15−17] and renal tubular cells.[18] The clinical use of phlorhizin has further been hampered by poor oral bioavailability and several adverse effects.[13] Nevertheless, phlorhizin has remained an important physiological tool to study the impact of SGLT1 on cell function and survival.[13,15−17]

Survival of erythrocytes critically depends on the availability of glucose,[19] cells taking up glucose by the passive glucose carrier GLUT1 and not by SGLT1.[20] Following glucose depletion, erythrocytes undergo suicidal death[19] or eryptosis, which is characterized by cell membrane scrambling and cell shrinkage.[21] Eryptosis is triggered by an increase of cytosolic Ca^{2+} concentration following Ca^{2+} entry through Ca^{2+}-permeable cation channels.[22−30] An increase of cytosolic Ca^{2+} concentration activates Ca^{2+}-sensitive K^+ channels[31,32] with subsequent cell shrinkage due to the exit of KCl together with osmotically obliged water.[33] Increased Ca^{2+} concentration further elicits cell membrane scrambling with exposure of phosphatidylserine at the cell surface.[30,34,35] Erythrocytes may be sensitized to the scrambling effect of Ca^{2+} by ceramide.[36] Ceramide formation is stimulated by platelet activating factor (PAF), which activates a sphingomyelinase leading to the breakdown of sphingomyelin.[37] Erythrocyte cell membrane scrambling could be further triggered by caspases, which are activated by oxidative stress.[35,38] The caspases are, however, not required for the scrambling effect of Ca^{2+}.[34,36,39]

The present study explored whether phlorhizin may influence eryptosis and, if it does, the identification of underlying mechanisms.

MATERIALS AND METHODS

Erythrocytes, Solutions, and Chemicals. Leukocyte-depleted erythrocytes were kindly provided by the blood bank of the University of Tübingen. The study is approved by the ethics committee of the University of Tübingen (184/2003 V).

Erythrocytes were incubated in vitro at a hematocrit of 0.4% in Ringer solution containing (in mM) 125 NaCl, 5 KCl, 1 $MgSO_4$, 32 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES), 5 glucose, and 1 $CaCl_2$ at pH 7.4 and 37 °C. As indicated, phlorhizin (Sigma, Freiburg, Germany) was added at the indicated concentrations, and *tert*-butylhydroperoxide (*t*-BOOH) was used at a concentration of 0.3 mM (Sigma).

FACS Analysis of Annexin V Binding and Forward Scatter. After incubation under the respective experimental condition, 50 μL cell suspensions were washed in Ringer solution containing 5 mM $CaCl_2$ and then stained with annexin V Fluos (1:500 dilutions; Roche, Mannheim, Germany) in this solution for 20 min under protection from light. In the following, the forward scatter (FSC) of the cells was determined, and annexin V fluorescence intensity was measured in FL-1 with an excitation wavelength of 488 nm and an emission wavelength of 530 nm on a FACS calibur (BD, Heidelberg, Germany).

Measurement of Intracellular Ca^{2+}. After incubation, a 50 μL erythrocyte suspension was washed in Ringer solution and then loaded with Fluo-3/AM (Calbiochem, Bad Soden, Germany) in Ringer solution containing 5 mM $CaCl_2$ and 2 μM Fluo-3/AM. The cells were incubated at 37 °C for 20 min and washed twice in Ringer solution containing 5 mM $CaCl_2$. The Fluo-3/AM-loaded erythrocytes were resuspended in 200 μL of Ringer. Then, Ca^{2+}-dependent fluorescence intensity was measured in fluorescence channel FL-1 on a FACS calibur (BD).

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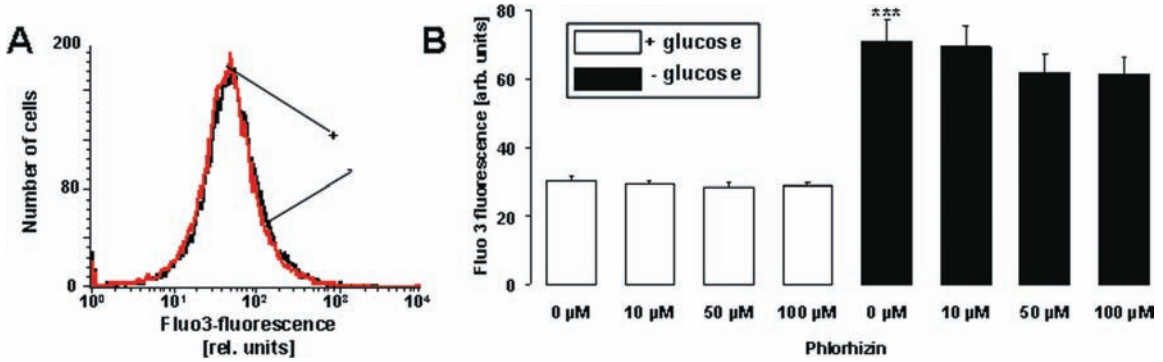

Figure 1. Effect of phlorhizin on erythrocyte cytosolic Ca^{2+} concentration following glucose depletion. (A) Original histogram of Fluo-3 fluorescence in erythrocytes following exposure for 48 h to Ringer solution without glucose, without (−, black line) and with the (+, red line) presence of 100 μM phlorhizin. (B) Arithmetic mean ± SEM ($n = 32$) of erythrocyte Fluo-3 fluorescence following exposure for 48 h to Ringer solution with (white bars) or without (black bars) glucose in the absence (0) or presence of 10−100 μM phlorhizin. *** ($p < 0.001$) indicates significant difference from the presence of glucose (ANOVA).

Estimation of the GSH/GSSG Ratio. Human erythrocytes (5% hematocrit) were incubated for 30 min at 37 °C in Ringer solution and in Ringer solution with 0.3 mM *tert*-butylhydroperoxide in the presence and absence of 100 μM phlorhizin. The cells were washed twice in PBS. All manipulations were performed on ice. After lysis of 50 μL of the erythrocyte pellet in 250 μL of distilled water and centrifugation at 14000 rpm, 150 μL of the supernatant was deproteinated by the addition of 150 μL of metaphosphoric acid (10%). Gluathione (GSSG and GSH) was measured with a glutathione assay kit (Cayman, Ann Arbor, MI) according to the manufacturer's protocol. The GSH/GSSG ratio refers to the concentrations within erythrocytes.

Determination of Intracellular ATP Concentration. For determination of intracellular erythrocyte ATP, 90 μL of erythrocyte pellets was incubated for 48 h at 37 °C in Ringer solution and in Ringer solution without glucose in the presence and absence of 100 μM phlorhizin (final hematocrit = 5%). All manipulations were then performed at 4 °C to avoid ATP degradation. Cells were lysed in distilled water, and proteins were precipitated by the addition of $HClO_4$ (5%). After centrifugation, an aliquot of the supernatant (400 μL) was adjusted to pH 7.7 by the addition of saturated $KHCO_3$ solution. After dilution of the supernatant, the ATP concentrations of the aliquots were determined utilizing the luciferin−luciferase assay kit (Roche Diagnostics) on a luminometer (Berthold Biolumat LB9500, Bad Wildbad, Germany) according to the manufacturer's protocol. ATP concentrations are expressed in millimoles per liter cytosol of erythrocytes.

Statistics. Data are expressed as the arithmetic mean ± SEM. Statistical analysis was made using paired ANOVA with Tukey's test as post-test, as appropriate. n denotes the number of different erythrocyte specimens studied. Because different erythrocyte specimens used in distinct experiments are differently susceptible to eryptotic effects, the same erythrocyte specimens have been used for control and experimental conditions.

RESULTS

To determine whether phlorhizin influences cytosolic Ca^{2+} concentration in erythrocytes, Fluo-3 fluorescence has been determined prior to and following glucose depletion in the absence and presence of phlorhizin. As shown in Figure 1, removal of glucose was followed by a marked increase of cytosolic Ca^{2+} concentration. Phlorhizin exposure did not significantly influence Fluo-3 fluorescence in the presence of glucose. Phlorhizin tended to slightly decrease Fluo-3 fluorescence during glucose depletion, an effect, however, not reaching statistical significance.

An increase of cytosolic Ca^{2+} concentration is known to stimulate K^+ channels with subsequent exit of KCl and cell shrinkage.[31−33] Accordingly, forward scatter was employed to estimate alterations of cell volume. As shown in Figure 2, exposure of erythrocytes for 48 h to glucose depletion was indeed followed by a sharp decrease of FSC. Phlorhizin did not significantly modify FSC in the presence of glucose, but blunted the decrease of FSC during glucose depletion, an effect reaching statistical significance at 50 μM phlorhizin (Figure 2).

An increase in cytosolic Ca^{2+} concentration is further known to stimulate cell membrane scrambling with phosphatidylserine exposure at the cell surface, which is identified by annexin V binding of the affected erythrocytes. As illustrated in Figure 3, the percentage of annexin V binding erythrocytes was markedly increased following exposure of erythrocytes for 48 h to glucose-free Ringer solution. Phlorhizin did not significantly modify annexin V binding in the presence of glucose, but significantly blunted the increase of annexin V binding following glucose depletion, an effect reaching statistical significance at 50 μM phlorhizin (Figure 3).

A further series of experiments explored whether phlorhizin affects the energy status of erythrocytes. In the presence of glucose the cytosolic ATP concentrations were not significantly different in the absence (1.0 ± 0.1 mM, $n = 4$) and in the presence (1.4 ± 0.1 mM, $n = 4$) of 100 μM phlorhizin. A 48 h exposure of erythrocytes to glucose depletion significantly reduced the cytosolic ATP content, an effect that was again not significantly different in the absence (0.6 ± 0.1 mM, $n = 4$) and in the presence (0.5 ± 0.1 mM, $n = 4$) of 100 μM phlorhizin.

Additional experiments were performed to explore whether phlorhizin interferes with the effect of oxidative stress on erythrocytes. As shown in Figure 4, exposure of erythrocytes to 0.3 mM *tert*-butylhydroperoxide was followed by a marked increase of cytosolic Ca^{2+} concentration. Phlorhizin exposure again did not significantly influence Fluo-3 fluorescence in the absence of oxidative stress. However, phlorhizin blunted the increase of Fluo-3 fluorescence following exposure of erythrocytes to 0.3 mM *tert*-butylhydroperoxide, an effect reaching statistical significance at 50 μM phlorhizin.

Oxidative stress further decreased cell volume. As shown in Figure 5, exposure of erythrocytes to 0.3 mM *tert*-butylhydroperoxide was followed by a decrease of forward scatter (Figure 5).

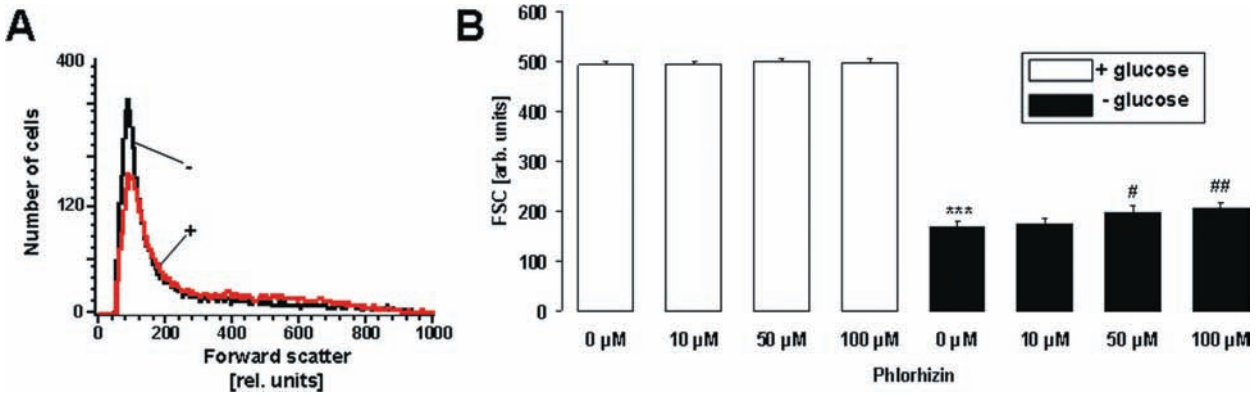

Figure 2. Effect of phlorhizin on erythrocyte forward scatter following glucose depletion. (A) Original histogram of forward scatter of erythrocytes following exposure for 48 h to Ringer solution without glucose, without (−, black line) and with the (+, red line) presence of 100 μM phlorhizin. (B) Arithmetic mean ± SEM (n = 32) of the erythrocyte forward scatter following incubation for 48 h in the presence (white bars) or absence (black bars) of glucose in the absence (0) or presence of 10−100 μM phlorhizin. *** (p < 0.001) indicates significant difference from the presence of glucose (ANOVA); # and ## (p < 0.05 and p < 0.01) indicate significant difference from the absence of phlorhizin (ANOVA).

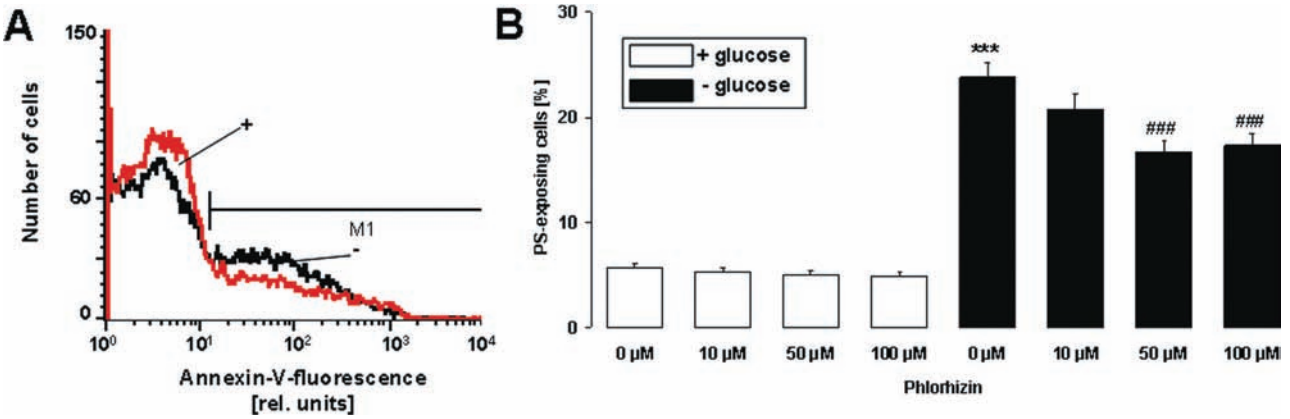

Figure 3. Effect of phlorhizin on phosphatidylserine exposure following glucose depletion. (A) Original histogram of annexin V binding of erythrocytes following exposure for 48 h to Ringer solution without glucose, without (−, black line) and with (+, red line) the presence of 100 μM phlorhizin. (B) Arithmetic mean ± SEM (n = 32) of erythrocyte annexin V binding following incubation for 48 h in the presence (white bars) or absence (black bars) of glucose in the absence (0) or presence of 10−100 μM phlorhizin. *** (p < 0.001) indicates significant difference from the presence of glucose (ANOVA); ### (p < 0.001) indicates significant difference from the absence of phlorhizin (ANOVA).

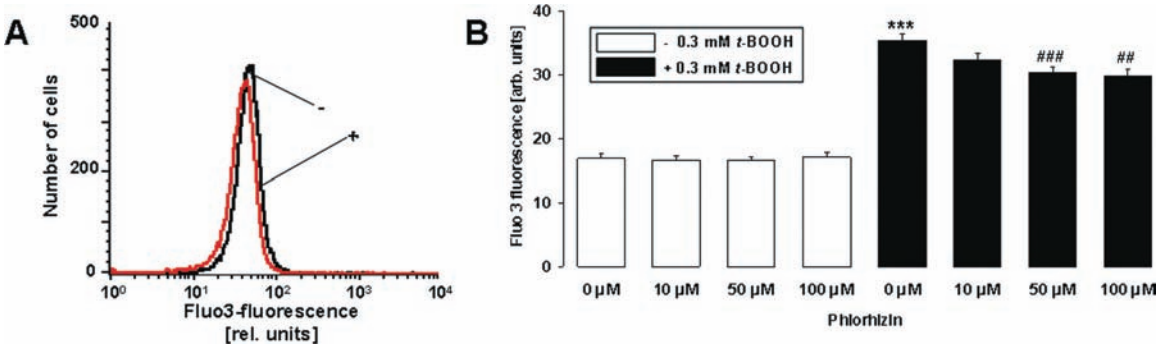

Figure 4. Effect of phlorhizin on erythrocyte cytosolic Ca^{2+} concentration following oxidative stress. (A) Original histogram of Fluo-3 fluorescence in erythrocytes following exposure for 30 min to Ringer solution with 0.3 mM *tert*-butylhydroperoxide, without (−, black line) and with (+, red line) the presence of 100 μM phlorhizin. (B) Arithmetic mean ± SEM (n = 28) of erythrocyte Fluo-3 fluorescence following exposure for 30 min to Ringer solution without (white bars, − 0.3 mM *t*-BOOH) or with (black bars, + 0.3 mM *t*-BOOH) 0.3 mM *tert*-butylhydroperoxide in the absence (0) or presence of 10−100 μM phlorhizin. *** (p < 0.001) indicates significant difference from the absence of 0.3 mM *tert*-butylhydroperoxide (ANOVA); ## and ### (p < 0.01 and p < 0.001) indicate significant difference from the absence of phlorhizin (ANOVA).

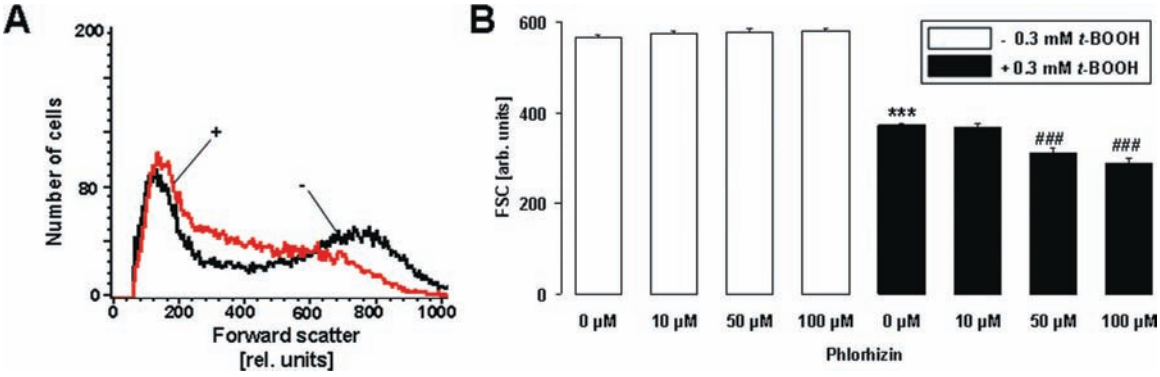

Figure 5. Effect of phlorhizin on erythrocyte forward scatter following oxidative stress. (A) Original histogram of forward scatter of erythrocytes following exposure for 30 min to Ringer solution with 0.3 mM *tert*-butylhydroperoxide, without (−, black line) and with (+, red line) the presence of 100 μM phlorhizin. (B) Arithmetic mean ± SEM (n = 28) of the erythrocyte forward scatter following exposure for 30 min to Ringer solution in the absence (white bars, − 0.3 mM *t*-BOOH) or presence (black bars, + 0.3 mM *t*-BOOH) of 0.3 mM *tert*-butylhydroperoxide in the absence (0) or presence of 10−100 μM phlorhizin. *** ($p < 0.001$) indicates significant difference from the absence of 0.3 mM *tert*-butylhydroperoxide (ANOVA); ### ($p < 0.001$) indicates significant difference from the absence of phlorhizin (ANOVA).

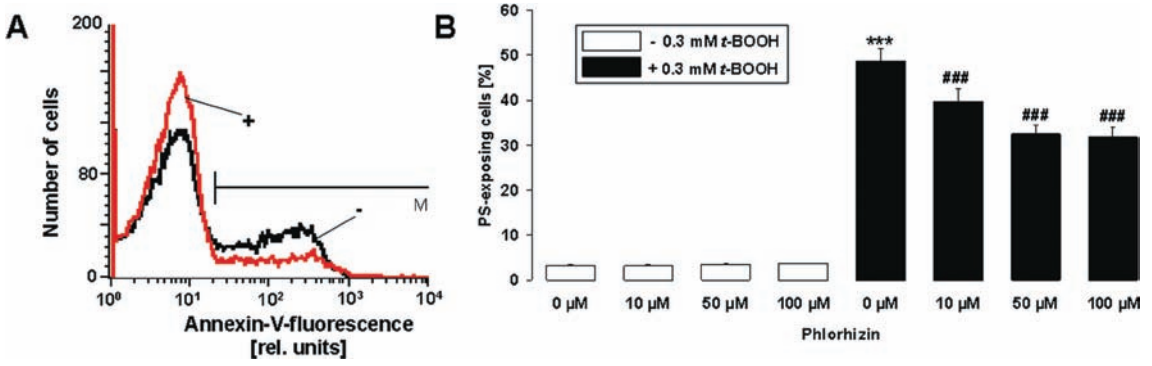

Figure 6. Effect of phlorhizin on phosphatidylserine exposure following oxidative stress. (A) Original histogram of annexin V binding of erythrocytes following exposure for 30 min to Ringer in the presence of 0.3 mM *tert*-butylhydroperoxide without (−, black line) and with (+, red line) the presence of 100 μM phlorhizin. (B) Arithmetic mean ± SEM (n = 28) of erythrocyte annexin V binding following exposure for 30 min to Ringer solution in the absence (white bars, − 0.3 mM *t*-BOOH) or presence (black bars, + 0.3 mM *t*-BOOH) of 0.3 mM *tert*-butylhydroperoxide in the absence (0) or presence of 10−100 μM phlorhizin. *** ($p < 0.001$) indicates significant difference from the absence of 0.3 mM tert-butylhydroperoxide (ANOVA); ### ($p < 0.001$) indicates significant difference from the absence of phlorhizin (ANOVA).

The addition of phlorhizin resulted in a further significant decrease of forward scatter.

According to annexin V binding, exposure of erythrocytes to 0.3 mM *tert*-butylhydroperoxide was followed by an increase of phosphatidylserine exposure (Figure 6), an effect again slightly but significantly blunted in the presence of phlorhizin (≥10 μM).

Additional experiments were performed to investigate whether phlorhizin affected the GSH/GSSG ratio in erythrocytes. In the absence of *tert*-butylhydroperoxide, the GSH/GSSG ratio approached 7.3 ± 1.6 (n = 4) in the absence of phlorhizin and 7.7 ± 1.2 (n = 4) in the presence of 100 μM phlorhizin. Following exposure to 0.3 mM *tert*-butylhydroperoxide the GSH/GSSG ratio declined to 3.1 ± 0.7 (n = 4) in the absence of phlorhizin and to 3.0 ± 0.3 (n = 4) in the presence of 100 μM phlorhizin. Accordingly, addition of 0.3 mM *tert*-butylhydroperoxide significantly decreased the GSH/GSSG ratio, an effect that was not significantly modified by 100 μM phlorhizin.

DISCUSSION

The present observations confirm the effect of glucose withdrawal and of *tert*-butylhydroperoxide exposure on cytosolic Ca^{2+} concentration, cell volume and cell membrane scrambling, all pointing to the stimulation of eryptosis by energy depletion and by oxidative stress.[21] More importantly, the present study reveals a subtle but significant blunting effect of phorhizin on eryptosis under energy depletion and oxidative stress. The concentrations needed for a significant effect on cell membrane scrambling were approximately 3-fold those approached in vivo following treatment of diabetic mice with a 0.5% phlorhizin diet, a dosage shown to counteract the hyperglycemia in streptozotocin-induced diabetic mice.[11] Following the 0.5% phlorhizin diet, metabolites of phlorhizin reached concentrations >1 magnitude higher than those of phlorhizin itself.[11] It remains to be shown whether those metabolites similarly affect erythrocyte survival.

The effect of phlorhizin on cytosolic Ca^{2+} activity is apparent only following oxidative stress. Presumably, under energy depletion the effect is too small to reach statistical significance. The Ca^{2+}-permeable cation channels in erythrocytes are activated by oxidative stress,[23] and energy depletion is similarly known to increase cytosolic Ca^{2+} activity.[19] The Ca^{2+}-permeable channels involve the transient receptor potential channel TRPC6.[25] How phlorhizin influences those channels cannot be derived from the present observations.

The increase of cytosolic Ca^{2+} activity activates Ca^{2+}-sensitive K^+ channels,[31,32] which have been identified as SK4.[40] The opening of the K^+ channels is followed by K^+ exit, which hyperpolarizes the cell, thus increasing the electrical driving force for Cl^- exit. The cellular loss of KCl with osmotically obliged water then decreases cell volume. Accordingly, Ca^{2+}-induced cell shrinkage is blunted in mice lacking SK4.[41] Phlorhizin blunts the cell shrinkage following glucose depletion, an effect presumably in part secondary to an inhibitory effect on Ca^{2+} entry. In any case, the effect cannot be explained by an influence on glucose transport. In contrast to its effect on cell volume in glucose depletion, phlorhizin augments the cell shrinkage following oxidative stress. It is tempting to speculate that oxidative stress triggers phlorhizin-sensitive uptake of glucose and/or other solutes, which counteracts the cell shrinkage due to activation of the K^+ channels.

An increase of cytosolic Ca^{2+} concentration further leads to stimulation of cell membrane scrambling. The effect of both energy depletion and oxidative stress on cell membrane scrambling is blunted by phlorhizin. The inhibitory effect of phlorhizin on cell membrane scrambling results at least in part from its effect on Ca^{2+} entry. Erythrocytes further express caspases,[35,38] which are activated by oxidative stress.[42] The caspases are known to cleave anion exchanger band 3[38] and stimulate phosphatidylserine exposure of erythrocytes.[38] However, other triggers of eryptosis, including energy depletion, do not require activation of caspases.[21] Thus, the effect of phlorhizin during energy depletion cannot be explained by inhibition of caspases.

In theory, phlorhizin or similar substances with more favorable pharmacological profile may be used to inhibit accelerated eryptosis. Disorders associated with accelerated eryptosis[21] include iron deficiency,[43] phosphate depletion,[44] hemolytic uremic syndrome,[45] sepsis,[46] sickle cell disease,[47] malaria,[37,48,49] and Wilson's disease.[50] Moreover, several xenobiotics and endogeneous substances stimulate eryptosis.[21,51−60]

A consequence of accelerated eryptosis is the development of anemia.[21] Moreover, phosphatidylserine-exposing erythrocytes may adhere to the vascular wall[61−65] and stimulate blood clotting.[61,66,67] Accordingly, enhanced eryptosis may interfere with microcirculation and contribute to the vascular injury of metabolic syndrome.[68]

In conclusion, the present study reveals a novel effect of phlorhizin, that is, blunting of eryptosis following energy depletion and following oxidative stress. Although the substance is not appropriate for use as drug, similar substances may prove to be useful in the treatment of disorders associated with excessive suicidal erythrocyte death.

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REFERENCES

(1) Bandoniene, D.; Murkovic, M. On-line HPLC-DPPH screening method for evaluation of radical scavenging phenols extracted from apples (*Malus domestica* L.). *J. Agric. Food Chem.* **2002**, *50*, 2482–2487.

(2) Hilt, P.; Schieber, A.; Yildirim, C.; Arnold, G.; Klaiber, I.; Conrad, J.; Beifuss, U.; Carle, R. Detection of phloridzin in strawberries (*Fragaria* × *ananassa* Duch.) by HPLC-PDA-MS/MS and NMR spectroscopy. *J. Agric. Food Chem.* **2003**, *51*, 2896–2899.

(3) Naz, S.; Siddiqi, R.; Dew, T. P.; Williamson, G. Epigallocatechin-3-gallate inhibits lactase but is alleviated by salivary proline-rich proteins. *J. Agric. Food Chem.* **2011**, *59*, 2734–2738.

(4) Pohl, C. H.; Kock, J. L.; Van Wyk, P. W.; Albertyn, J. *Cryptococcus anemochoreius* sp. nov., a novel anamorphic basidiomycetous yeast isolated from the atmosphere in central South Africa. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 2703–2706.

(5) Turner, A.; Chen, S. N.; Joike, M. K.; Pendland, S. L.; Pauli, G. F.; Farnsworth, N. R. Inhibition of uropathogenic *Escherichia coli* by cranberry juice: a new antiadherence assay. *J. Agric. Food Chem.* **2005**, *53*, 8940–8947.

(6) Xu, X.; Xie, H.; Hao, J.; Jiang, Y.; Wei, X. Flavonoid glycosides from the seeds of *Litchi chinensis*. *J. Agric. Food Chem.* **2011**, *59*, 1205–1209.

(7) Gosch, C.; Halbwirth, H.; Stich, K. Phloridzin: biosynthesis, distribution and physiological relevance in plants. *Phytochemistry* **2010**, *71*, 838–843.

(8) Henry, C.; Vitrac, X.; Decendit, A.; Ennamany, R.; Krisa, S.; Merillon, J. M. Cellular uptake and efflux of *trans*-piceid and its aglycone *trans*-resveratrol on the apical membrane of human intestinal Caco-2 cells. *J. Agric. Food Chem.* **2005**, *53*, 798–803.

(9) Hummel, C. S.; Lu, C.; Loo, D. D.; Hirayama, B. A.; Voss, A. A.; Wright, E. M. Glucose transport by human renal Na^+/D-glucose cotransporters SGLT1 and SGLT2. *Am. J. Physiol. Cell Physiol.* **2011**, *300*, C14–C21.

(10) Kinne, R. K.; Castaneda, F. SGLT inhibitors as new therapeutic tools in the treatment of diabetes. *Handb. Exp. Pharmacol.* **2011**, 105–126.

(11) Masumoto, S.; Akimoto, Y.; Oike, H.; Kobori, M. Dietary phloridzin reduces blood glucose levels and reverses SGLT1 expression in the small intestine in streptozotocin-induced diabetic mice. *J. Agric. Food Chem.* **2009**, *57*, 4651–4656.

(12) Mishima, T.; Tanaka, K.; Tsuge, H.; Sugita, J.; Nakahara, M.; Hayakawa, T. Studies on absorption and hydrolysis of ethyl α-D-glucoside in rat intestine. *J. Agric. Food Chem.* **2005**, *53*, 7257–7261.

(13) Mather, A.; Pollock, C. Renal glucose transporters: novel targets for hyperglycemia management. *Nat. Rev. Nephrol* **2010**, *6*, 307–311.

(14) Malatiali, S.; Francis, I.; Barac-Nieto, M. Phlorizin prevents glomerular hyperfiltration but not hypertrophy in diabetic rats. *Exp. Diabetes Res.* **2008**, *2008*, 305403.

(15) Huang, C. Y.; Hsiao, J. K.; Lu, Y. Z.; Lee, T. C.; Yu, L. C. Anti-apoptotic PI3K/Akt signaling by sodium/glucose transporter 1 reduces epithelial barrier damage and bacterial translocation in intestinal ischemia. *Lab. Invest.* **2011**, *91*, 294–309.

(16) Yu, L. C.; Flynn, A. N.; Turner, J. R.; Buret, A. G. SGLT-1-mediated glucose uptake protects intestinal epithelial cells against LPS-induced apoptosis and barrier defects: a novel cellular rescue mechanism? *FASEB J.* **2005**, *19*, 1822–1835.

(17) Yu, L. C.; Huang, C. Y.; Kuo, W. T.; Sayer, H.; Turner, J. R.; Buret, A. G. SGLT-1-mediated glucose uptake protects human intestinal epithelial cells against *Giardia duodenalis*-induced apoptosis. *Int. J. Parasitol.* **2008**, *38*, 923–934.

(18) Ikari, A.; Nagatani, Y.; Tsukimoto, M.; Harada, H.; Miwa, M.; Takagi, K. Sodium-dependent glucose transporter reduces peroxynitrite and cell injury caused by cisplatin in renal tubular epithelial cells. *Biochim. Biophys. Acta* **2005**, *1717*, 109–117.

(19) Klarl, B. A.; Lang, P. A.; Kempe, D. S.; Niemoeller, O. M.; Akel, A.; Sobiesiak, M.; Eisele, K.; Podolski, M.; Huber, S. M.; Wieder, T.; Lang, F. Protein kinase C mediates erythrocyte "programmed cell death" following glucose depletion. *Am. J. Physiol. Cell Physiol.* **2006**, *290*, C244–C253.

(20) Takata, K.; Kasahara, T.; Kasahara, M.; Ezaki, O.; Hirano, H. Localization of Na(+)-dependent active type and erythrocyte/HepG2-type glucose transporters in rat kidney: immunofluorescence and immunogold study. *J. Histochem. Cytochem.* **1991**, *39*, 287–298.

(21) Lang, F.; Gulbins, E.; Lerche, H.; Huber, S. M.; Kempe, D. S.; Föller, M. Eryptosis, a window to systemic disease. *Cell Physiol. Biochem.* **2008**, *22*, 373–380.

(22) Bernhardt, I.; Weiss, E.; Robinson, H. C.; Wilkins, R.; Bennekou, P. Differential effect of HOE642 on two separate monovalent cation transporters in the human red cell membrane. *Cell Physiol. Biochem.* **2007**, *20*, 601–606.

(23) Duranton, C.; Huber, S. M.; Lang, F. Oxidation induces a Cl(−)-dependent cation conductance in human red blood cells. *J. Physiol* **2002**, *539*, 847–855.

(24) Duranton, C.; Huber, S.; Tanneur, V.; Lang, K.; Brand, V.; Sandu, C.; Lang, F. Electrophysiological properties of the *Plasmodium falciparum*-induced cation conductance of human erythrocytes. *Cell Physiol. Biochem.* **2003**, *13*, 189–198.

(25) Foller, M.; Kasinathan, R. S.; Koka, S.; Lang, C.; Shumilina, E.; Birnbaumer, L.; Lang, F.; Huber, S. M. TRPC6 contributes to the Ca(2+) leak of human erythrocytes. *Cell Physiol. Biochem.* **2008**, *21*, 183–192.

(26) Foller, M.; Sopjani, M.; Koka, S.; Gu, S.; Mahmud, H.; Wang, K.; Floride, E.; Schleicher, E.; Schulz, E.; Munzel, T.; Lang, F. Regulation of erythrocyte survival by AMP-activated protein kinase. *FASEB J.* **2009**, *23*, 1072–1080.

(27) Huber, S. M.; Gamper, N.; Lang, F. Chloride conductance and volume-regulatory nonselective cation conductance in human red blood cell ghosts. *Pfluegers Arch.* **2001**, *441*, 551–558.

(28) Kaestner, L.; Christophersen, P.; Bernhardt, I.; Bennekou, P. The non-selective voltage-activated cation channel in the human red blood cell membrane: reconciliation between two conflicting reports and further characterisation. *Bioelectrochemistry* **2000**, *52*, 117–125.

(29) Kaestner, L.; Bernhardt, I. Ion channels in the human red blood cell membrane: their further investigation and physiological relevance. *Bioelectrochemistry* **2002**, *55*, 71–74.

(30) Lang, K. S.; Duranton, C.; Poehlmann, H.; Myssina, S.; Bauer, C.; Lang, F.; Wieder, T.; Huber, S. M. Cation channels trigger apoptotic death of erythrocytes. *Cell Death Differ.* **2003**, *10*, 249–256.

(31) Bookchin, R. M.; Ortiz, O. E.; Lew, V. L. Activation of calcium-dependent potassium channels in deoxygenated sickled red cells. *Prog. Clin. Biol. Res.* **1987**, *240*, 193–200.

(32) Brugnara, C.; de Franceschi, L.; Alper, S. L. Inhibition of Ca(2+)-dependent K+ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. *J. Clin. Invest.* **1993**, *92*, 520–526.

(33) Lang, P. A.; Kaiser, S.; Myssina, S.; Wieder, T.; Lang, F.; Huber, S. M. Role of Ca^{2+}-activated K^+ channels in human erythrocyte apoptosis. *Am. J. Physiol. Cell Physiol.* **2003**, *285*, C1553–C1560.

(34) Berg, C. P.; Engels, I. H.; Rothbart, A.; Lauber, K.; Renz, A.; Schlosser, S. F.; Schulze-Osthoff, K.; Wesselborg, S. Human mature red blood cells express caspase-3 and caspase-8, but are devoid of mitochondrial regulators of apoptosis. *Cell Death Differ.* **2001**, *8*, 1197–1206.

(35) Bratosin, D.; Estaquier, J.; Petit, F.; Arnoult, D.; Quatannens, B.; Tissier, J. P.; Slomianny, C.; Sartiaux, C.; Alonso, C.; Huart, J. J.; Montreuil, J.; Ameisen, J. C. Programmed cell death in mature erythrocytes: a model for investigating death effector pathways operating in the absence of mitochondria. *Cell Death Differ.* **2001**, *8*, 1143–1156.

(36) Lang, K. S.; Myssina, S.; Brand, V.; Sandu, C.; Lang, P. A.; Berchtold, S.; Huber, S. M.; Lang, F.; Wieder, T. Involvement of ceramide in hyperosmotic shock-induced death of erythrocytes. *Cell Death Differ.* **2004**, *11*, 231–243.

(37) Lang, P. A.; Kempe, D. S.; Tanneur, V.; Eisele, K.; Klarl, B. A.; Myssina, S.; Jendrossek, V.; Ishii, S.; Shimizu, T.; Waidmann, M.; Hessler, G.; Huber, S. M.; Lang, F.; Wieder, T. Stimulation of erythrocyte ceramide formation by platelet-activating factor. *J. Cell Sci.* **2005**, *118*, 1233–1243.

(38) Mandal, D.; Moitra, P. K.; Saha, S.; Basu, J. Caspase 3 regulates phosphatidylserine externalization and phagocytosis of oxidatively stressed erythrocytes. *FEBS Lett.* **2002**, *513*, 184–188.

(39) Weil, M.; Jacobson, M. D.; Raff, M. C. Are caspases involved in the death of cells with a transcriptionally inactive nucleus? Sperm and chicken erythrocytes. *J. Cell Sci.* **1998**, *111* (Part 18), 2707–2715.

(40) Hoffman, J. F.; Joiner, W.; Nehrke, K.; Potapova, O.; Foye, K.; Wickrema, A. The hSK4 (KCNN4) isoform is the Ca^{2+}-activated K^+ channel (Gardos channel) in human red blood cells. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7366–7371.

(41) Foller, M.; Bobbala, D.; Koka, S.; Boini, K. M.; Mahmud, H.; Kasinathan, R. S.; Shumilina, E.; Amann, K.; Beranek, G.; Sausbier, U.; Ruth, P.; Sausbier, M.; Lang, F.; Huber, S. M. Functional significance of the intermediate conductance Ca^{2+}-activated K^+ channel for the short-term survival of injured erythrocytes. *Pfluegers Arch.* **2010**, *460*, 1029–1044.

(42) Matarrese, P.; Straface, E.; Pietraforte, D.; Gambardella, L.; Vona, R.; Maccaglia, A.; Minetti, M.; Malorni, W. Peroxynitrite induces senescence and apoptosis of red blood cells through the activation of aspartyl and cysteinyl proteases. *FASEB J.* **2005**, *19*, 416–418.

(43) Kempe, D. S.; Lang, P. A.; Duranton, C.; Akel, A.; Lang, K. S.; Huber, S. M.; Wieder, T.; Lang, F. Enhanced programmed cell death of iron-deficient erythrocytes. *FASEB J.* **2006**, *20*, 368–370.

(44) Birka, C.; Lang, P. A.; Kempe, D. S.; Hoefling, L.; Tanneur, V.; Duranton, C.; Nammi, S.; Henke, G.; Myssina, S.; Krikov, M.; Huber, S. M.; Wieder, T.; Lang, F. Enhanced susceptibility to erythrocyte "apoptosis" following phosphate depletion. *Pfluegers Arch.* **2004**, *448*, 471–477.

(45) Lang, P. A.; Beringer, O.; Nicolay, J. P.; Amon, O.; Kempe, D. S.; Hermle, T.; Attanasio, P.; Akel, A.; Schafer, R.; Friedrich, B.; Risler, T.; Baur, M.; Olbricht, C. J.; Zimmerhackl, L. B.; Zipfel, P. F.; Wieder, T.; Lang, F. Suicidal death of erythrocytes in recurrent hemolytic uremic syndrome. *J. Mol. Med.* **2006**, *84*, 378–388.

(46) Kempe, D. S.; Akel, A.; Lang, P. A.; Hermle, T.; Biswas, R.; Muresanu, J.; Friedrich, B.; Dreischer, P.; Wolz, C.; Schumacher, U.; Peschel, A.; Gotz, F.; Doring, G.; Wieder, T.; Gulbins, E.; Lang, F. Suicidal erythrocyte death in sepsis. *J. Mol. Med.* **2007**, *85*, 269–277.

(47) Lang, P. A.; Kasinathan, R. S.; Brand, V. B.; Duranton, C.; Lang, C.; Koka, S.; Shumilina, E.; Kempe, D. S.; Tanneur, V.; Akel, A.; Lang, K. S.; Foller, M.; Kun, J. F.; Kremsner, P. G.; Wesselborg, S.; Laufer, S.; Clemen, C. S.; Herr, C.; Noegel, A. A.; Wieder, T.; Gulbins, E.; Lang, F.; Huber, S. M. Accelerated clearance of *Plasmodium*-infected erythrocytes in sickle cell trait and annexin-A7 deficiency. *Cell Physiol. Biochem.* **2009**, *24*, 415–428.

(48) Foller, M.; Bobbala, D.; Koka, S.; Huber, S. M.; Gulbins, E.; Lang, F. Suicide for survival — death of infected erythrocytes as a host mechanism to survive malaria. *Cell Physiol. Biochem.* **2009**, *24*, 133–140.

(49) Koka, S.; Bobbala, D.; Lang, C.; Boini, K. M.; Huber, S. M.; Lang, F. Influence of paclitaxel on parasitemia and survival of *Plasmodium berghei* infected mice. *Cell Physiol. Biochem.* **2009**, *23*, 191–198.

(50) Lang, P. A.; Schenck, M.; Nicolay, J. P.; Becker, J. U.; Kempe, D. S.; Lupescu, A.; Koka, S.; Eisele, K.; Klarl, B. A.; Rubben, H.; Schmid, K. W.; Mann, K.; Hildenbrand, S.; Hefter, H.; Huber, S. M.; Wieder, T.; Erhardt, A.; Haussinger, D.; Gulbins, E.; Lang, F. Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. *Nat. Med.* **2007**, *13*, 164–170.

(51) Bhavsar, S. K.; Eberhard, M.; Bobbala, D.; Lang, F. Monensin induced suicidal erythrocyte death. *Cell Physiol. Biochem.* **2010**, *25*, 745–752.

(52) Bhavsar, S. K.; Bobbala, D.; Xuan, N. T.; Foller, M.; Lang, F. Stimulation of suicidal erythrocyte death by alpha-lipoic acid. *Cell Physiol. Biochem.* **2010**, *26*, 859–868.

(53) Bobbala, D.; Alesutan, I.; Foller, M.; Huber, S. M.; Lang, F. Effect of anandamide in *Plasmodium berghei*-infected mice. *Cell Physiol. Biochem.* **2010**, *26*, 355–362.

(54) Eberhard, M.; Ferlinz, K.; Alizzi, K.; Cacciato, P. M.; Faggio, C.; Foller, M.; Lang, F. FTY720-induced suicidal erythrocyte death. *Cell Physiol. Biochem.* **2010**, *26*, 761–766.

(55) Siraskar, B.; Ballal, A.; Bobbala, D.; Foller, M.; Lang, F. Effect of amphotericin B on parasitemia and survival of *Plasmodium berghei*-infected mice. *Cell Physiol. Biochem.* **2010**, *26*, 347–354.

(56) Braun, M.; Foller, M.; Gulbins, E.; Lang, F. Eryptosis triggered by bismuth. *Biometals* **2009**, *22*, 453–460.

(57) Mahmud, H.; Foller, M.; Lang, F. Arsenic-induced suicidal erythrocyte death. *Arch. Toxicol.* **2009**, *83*, 107–113.

(58) Mahmud, H.; Mauro, D.; Qadri, S. M.; Foller, M.; Lang, F. Triggering of suicidal erythrocyte death by amphotericin B. *Cell Physiol. Biochem.* **2009**, *24*, 263–270.

(59) Mahmud, H.; Mauro, D.; Foller, M.; Lang, F. Inhibitory effect of thymol on suicidal erythrocyte death. *Cell Physiol. Biochem.* **2009**, *24*, 407–414.

(60) Nguyen, T. T.; Foller, M.; Lang, F. Tin triggers suicidal death of erythrocytes. *J. Appl. Toxicol.* **2008**, *29*, 79–83.

(61) Andrews, D. A.; Low, P. S. Role of red blood cells in thrombosis. *Curr. Opin. Hematol.* **1999**, *6*, 76–82.

(62) Closse, C.; Dachary-Prigent, J.; Boisseau, M. R. Phosphatidylserine-related adhesion of human erythrocytes to vascular endothelium. *Br. J. Hamaetol.* **1999**, *107*, 300–302.

(63) Gallagher, P. G.; Chang, S. H.; Rettig, M. P.; Neely, J. E.; Hillery, C. A.; Smith, B. D.; Low, P. S. Altered erythrocyte endothelial adherence and membrane phospholipid asymmetry in hereditary hydrocytosis. *Blood* **2003**, *101*, 4625–4627.

(64) Pandolfi, A.; Di Pietro, N.; Sirolli, V.; Giardinelli, A.; Di Silvestre, S.; Amoroso, L.; Di Tomo, P.; Capani, F.; Consoli, A.; Bonomini, M. Mechanisms of uremic erythrocyte-induced adhesion of human monocytes to cultured endothelial cells. *J. Cell Physiol.* **2007**, *213*, 699–709.

(65) Wood, B. L.; Gibson, D. F.; Tait, J. F. Increased erythrocyte phosphatidylserine exposure in sickle cell disease: flow-cytometric measurement and clinical associations. *Blood* **1996**, *88*, 1873–1880.

(66) Chung, S. M.; Bae, O. N.; Lim, K. M.; Noh, J. Y.; Lee, M. Y.; Jung, Y. S.; Chung, J. H. Lysophosphatidic acid induces thrombogenic activity through phosphatidylserine exposure and procoagulant microvesicle generation in human erythrocytes. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 414–421.

(67) Zwaal, R. F.; Comfurius, P.; Bevers, E. M. Surface exposure of phosphatidylserine in pathological cells. *Cell. Mol. Life Sci.* **2005**, *62*, 971–988.

(68) Zappulla, D. Environmental stress, erythrocyte dysfunctions, inflammation, and the metabolic syndrome: adaptations to CO_2 increases? *J. Cardiometab. Syndr.* **2008**, *3*, 30–34.