Chronic hypoxia alters glucose utilization during GSH-dependent detoxication in rat small intestine

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LeGrand, Terry S., and Tak Yee Aw. Chronic hypoxia alters glucose utilization during GSH-dependent detoxication in rat small intestine. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G376–G384, 1998.—We showed that hypoxia alters glutathione (GSH)-dependent detoxication and induces mucosal metabolic instability. To determine the impact of these changes and the role of reductant supply in intestinal lipid peroxide disposition, pair-fed (16 g/day) Sprague-Dawley rats were exposed to air (20.9% O₂; n = 6) or 10% O₂ (n = 6) for 10 days. Jejunal and ileal everted sacs were exposed to 75 µM peroxidized fish oil with or without 10 mM glucose or 1 mM GSH. Peroxide transport was determined as the abluminal recovery of thiobarbituric acid-reactive substances. Peroxide recovery in hypoxic intestine was twice that in normoxic intestine. Addition of GSH and glucose did not affect peroxide recovery, indicating reduced intracellular GSH-dependent metabolism and enhanced output by the hypoxic intestine. Glucose uptake by normoxic and hypoxic intestine is similar, whereas its utilization for detoxication is decreased in hypoxic cells. Determination of NADPH supply indicates that decreased glucose availability for NADPH production during hypoxia impairs GSH disulfide reduction, compromises hydroperoxide metabolism, and increases peroxide output from hypoxic intestine.

chronic O₂ deficiency is a common clinical condition in disease states such as obstructive lung disease and cardiovascular insufficiency (10, 31). Chronic hypoxia has been associated with a decrease in total plasma protein content (16), as well as an overall reduction in protein synthesis (13). The intestine and other tissues rely on an adequate O₂ supply for optimal function (2, 17, 19). Previous studies have shown that chronic O₂ deficiency impairs renal function (18), alters nutrient (23) and drug absorption (4), and decreases gastric emptying, which may impact pharmacodynamics in drug therapy regimens (33). In recent studies, Bai and Jones (7) showed that glutathione (GSH) transport by intestinal cells is inhibited by chronic hypoxia, and the rate of peroxidized methyl linoleate uptake is decreased in hypoxic compared with normoxic intestine. We (21, 22) have shown in our laboratory that chronic hypoxia promotes intestinal oxidative stress, induces mucosal metabolic instability, and compromises GSH-dependent detoxication in the intestine. Because GSH is an important component of peroxide metabolism in the intestine (3), these findings suggest that disposition of toxic peroxides may be compromised in the hypoxic intestine.

Lipid peroxides are present in our diets to varying degrees. One source of lipid peroxides in the diet is foods that are high in oxidizable polyunsaturated fats (1). Fats comprise up to 40% of the calories in the American diet (15, 20), an appreciable amount of which is oxidized (29); thus the gastrointestinal tract must be equipped with an efficient detoxication system to maintain its function as an interface between ingested substances and the body. An important peroxide detoxication pathway in the intestine is the GSH redox system (Fig. 1). In this system GSH peroxidase reduces peroxides at the expense of GSH, with concomitant production of glutathione disulfide (GSSG). GSSG is converted to its reduced form by GSSG reductase, utilizing NADPH as a reductant. A major source of NADPH in the intestine is the pentose phosphate pathway (35), which is regulated by glucose flux (3).

We previously found that luminal uptake (absorption) and transintestinal output (transport into lymph) of lipid peroxides by the small intestine is dependent on function of the GSH redox cycle (5, 6). Our hypothesis is conceptually illustrated in Fig. 2. In the GSH-sufficient state, the intracellular catabolism of hydroperoxides by the GSH redox cycle drives the absorption of peroxides from the lumen via a large lumen-to-cell gradient, a form of metabolic trapping. The result is decreased hydroperoxide retention in the lumen as well as decreased output into lymph. The GSH-deficient state, in which intracellular peroxide catabolism is impaired, results in diminished peroxide absorption from the gut lumen and enhanced peroxide output into lymph as a result of decreased intracellular peroxide metabolism. Given that chronic hypoxia compromises GSH-dependent pathways, it is likely that luminal lipid peroxide disposition and peroxide transport are impaired in the hypoxic intestine. Elevated plasma peroxides may have implications for systemic pathologies, such as atherosclerosis (8, 27). The purpose of the current study is to determine the impact of prolonged O₂ deficiency on intestinal lipid peroxide metabolism and disposition and to define the contribution of glucose and reductant supply (GSH and NADPH) to peroxide detoxication in the hypoxic intestine.

METHODS

Induction of chronic hypoxia. Male Sprague-Dawley rats weighing 300–350 g were exposed to either normoxia (20.9% O₂) or hypoxia (10% O₂) in plastic cages for 10 days as previously described (4). This protocol induces moderately severe, but not life-threatening, hypoxia. Brieﬂy, the desired P O₂ was achieved by using air (normoxia) or by combining air and nitrogen in a Matheson gas mixer. The P O₂ in the chambers was monitored with a Clark-type O₂ electrode inserted through a small opening in the cover. Because hypoxic animals exhibit decreased food intake, normoxic controls were fed equivalent amounts (16 g/day), in contrast...
conditions. The iodometric method involves the specific reduction of lipid hydroperoxides under these experimental conditions. The iodometric method (9) that the TBA assay predominantly measures lipid hydroperoxides and/or cyclic endoperoxides, with TBA was measured at 532 nm, and it correlates well with values obtained with the TBA assay, indicating that this assay can be used to quantify lipid hydroperoxides in the current study. Furthermore, high-performance liquid chromatography (HPLC) analyses revealed that the lipid hydroperoxides are predominantly those of hydroperoxyeicosapentaenoic (20:5) and docosahexaenoic (22:6) acids (11). A 20% homogenate was prepared from samples of tissues after exposure to peroxidized fish oil, and samples were taken for determinations of GSH and protein thiol concentrations.

Cell isolation and measurement of glucose uptake. Jejunum and ileal enterocytes were isolated from rat intestine according to the method of Masola and Evered (24). Briefly, rats were killed under halothane anesthesia, and the first 5 cm of intestine distal to the stomach (duodenum) were discarded. The remaining small intestine was divided, with the proximal portion taken as jejunum and the distal portion as ileum. The lumen was washed with cold 0.9% saline solution to remove particulate matter. The lumen was then filled with Krebs-Henseleit buffer, pH 7.4, containing 10 mM dithiothreitol (DTT), and incubated for 10 min at 37°C to remove excess mucus. The lumen was refilled with buffer containing 5 mM ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetate (EGTA) and 10 mM DTT and incubated for an additional 15 min at 37°C. Segments were massaged gently to release enterocytes, which were filtered through two layers of gauze and washed in buffer containing 10 mM DTT and 0.25% bovine serum albumin (BSA). Cells were resuspended in DTT- and BSA-free buffer at a concentration of 5.0 × 10⁶ cells/ml. Hypoxic enterocytes were exposed to atmospheric O₂ tension during these experiments, but we have previously shown that acute exposure of hypoxic cells to environmental O₂ tension does not cause metabolic properties of the cells to revert to those of normal cells for at least 48 h (4). Cells were incubated with 10 mM [¹⁴C]glucose for 30 min. At designated times, samples were taken and added to ice-cold enterocyte isolation buffer and filtered through a 47-mm, 5-µm polycarbonate filter. Cell-associated [¹⁴C]glucose was measured using a Wallac model 1409 liquid scintillation counter. Glucose uptake is expressed as nanomoles per 10⁶ cells.

Diamide-infusion into enterocyte suspensions. Enterocytes (4–5 × 10⁹/ml) were incubated in 5-ml rotating round-bottom flasks at 37°C, without or with added 10 mM glucose. Diamide-containing solutions at different concentrations were infused at a rate of 1 ml/h into the cell suspensions with the use of a peristaltic pump (Pharmacia LKB Biotechnology, Piscataway, NJ), equipped with 2-mm ID tubing as previously described (32). Diamide concentrations were varied to give infusion rates of 0.01–0.8 nmol · min⁻¹ · 10⁶ cells⁻¹. Incubations were performed for 15 min, with samples taken at various time points. Our laboratory has previously shown that there is no difference in cell viability at the different diamide doses, and at the low infusion rate the maximum dilution of cell suspensions was 3% (3). At designated time points, 0.5 ml of cell suspension was removed and the cells were separated from the incubation mixture by centrifugation. The acid supernatants were assayed for GSH and GSSG.

Biochemical assays. TBARS were measured using the method of Buege and Aust (9). Determination of lipid hydroperoxides using the TBARS method was correlated with that using the iodometric method (9) and HPLC (11). GSH concentration was determined spectrophotometrically by the method of Owens and Belcher (26), and by HPLC according to Reed et al. (28), which allowed measurement of GSH and GSSG. Protein thiols were measured according to the method of Ellman and Lysko (14).
Effect of chronic hypoxia on intestinal transport of peroxide. Figure 3 shows peroxide transport by normoxic and hypoxic ileum over 30 min. A previous study in our laboratory showed a decreasing proximal-to-distal gradient of intestinal GSH reductase cycle enzymes (21), suggesting more pronounced impairment of detoxification capacity in ileal intestine. Data from the ileal intestine are shown graphically, whereas those from the jejunum are given in tables. The results in Fig. 3 show that in ad libitum-fed and pair-fed normoxic controls, ileal peroxide transport over the experimental period was minimal. Although hypoxic ileal enterocytes initially transported negligible amounts of peroxide as well, after 10-min exposure to peroxidized lipids, hypoxic enterocytes began to transport increasing quantities of peroxide into the contraluminal compartment. At 20 and 30 min, hypoxic cells had transported significantly more peroxide than pair-fed or ad libitum-fed normoxic controls. When hydroperoxide transport over 30 min is expressed as a percentage of that made available to intestinal segments in the incubation medium (1,125 nmol in 15 ml volume), intestine from ad libitum-fed and pair-fed normoxic rats transported about 5% of the available hydroperoxides per gram of tissue, whereas hypoxic intestine transported about 13% per gram of tissue. Results in ad libitum- and pair-fed normoxic controls are similar in this and subsequent studies; therefore, data are limited to results from pair-fed normoxic and chronically hypoxic animals. Response of jejunal enterocytes to hydroperoxide exposure was similar to that of ileal enterocytes (Table 1).

Effect of exogenous glucose and GSH on peroxide transport and tissue peroxide retention. Figure 4A shows peroxide transport by normoxic and hypoxic ileal intestine in the absence or presence of 10 mM glucose and/or 1 mM GSH over 30 min. Before exposure to peroxidized lipids, peroxide transport by normoxic and hypoxic ileum is minimal. In the absence of substrates (glucose and/or GSH), contraluminal peroxide recovery in hypoxic ileum was significantly higher than in normoxic ileum. Transport of peroxide is attenuated in the presence of exogenous glucose and GSH in normoxic and hypoxic ileum over 30 min. A previous study in our laboratory showed a decreasing proximal-to-distal gradient of intestinal GSH reductase cycle enzymes (21), suggesting more pronounced impairment of detoxification capacity in ileal intestine. Data from the ileal intestine are shown graphically, whereas those from the jejunum are given in tables. The results in Fig. 3 show that in ad libitum-fed and pair-fed normoxic controls, ileal peroxide transport over the experimental period was minimal. Although hypoxic ileal enterocytes initially transported negligible amounts of peroxide as well, after 10-min exposure to peroxidized lipids, hypoxic enterocytes began to transport increasing quantities of peroxide into the contraluminal compartment. At 20 and 30 min, hypoxic cells had transported significantly more peroxide than pair-fed or ad libitum-fed normoxic controls. When hydroperoxide transport over 30 min is expressed as a percentage of that made available to intestinal segments in the incubation medium (1,125 nmol in 15 ml volume), intestine from ad libitum-fed and pair-fed normoxic rats transported about 5% of the available hydroperoxides per gram of tissue, whereas hypoxic intestine transported about 13% per gram of tissue. Results in ad libitum- and pair-fed normoxic controls are similar in this and subsequent studies; therefore, data are limited to results from pair-fed normoxic and chronically hypoxic animals. Response of jejunal enterocytes to hydroperoxide exposure was similar to that of ileal enterocytes (Table 1).

Effect of chronic hypoxia on the transport of peroxidized lipids by ileal enterocytes. Everted intestinal sacs containing Krebs-Henseleit buffer were incubated for 0–30 min at 37°C in 75 µM peroxidized lipid solution. At designated times, intestinal sacs were removed and peroxide transport was determined as the contraluminal recovery of thiobarbituric acid-reactive substances (TBARS). Data are means ± SE; n = 3 everted sac preparations for ad libitum-fed (○) and pair-fed normoxic controls (●), and n = 4 everted sac preparations for chronically hypoxic animals (□). *P < 0.05 vs. ad libitum-fed and pair-fed normoxic controls.

Statistical analysis. Data are expressed as means ± SE. Analysis of variance and Student's t-test were used to determine significance of differences. P < 0.05 was considered significant.

Materials. DTT, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, EGTA, BSA, TBA, GSH, taurocholic acid, trichloroacetic acid, 2,4-dinitrofluorobenzene (Sanger's reagent), 5,5'-dithio-bis(2-nitrobenzoic acid) (Ellman's reagent), N-ethylmaleimide, and Triton X-100 were purchased from Sigma Chemical (St. Louis, MO). [14C]glucose was purchased from ICN Pharmaceuticals (Costa Mesa, CA). All other chemicals were of reagent grade and were available locally.
Hypoxic conditions, whereas addition of these substrates did not affect abluminal peroxide recovery in hypoxic intestine. This finding signifies the presence of reduced intracellular peroxide metabolism and enhanced output from the hypoxic intestine. Peroxide transport data from normoxic and hypoxic jejenum are shown in Table 1. Intraluminal peroxide recovery in hypoxic jejunum was significantly higher than in normoxic jejunum at 20 and 30 min and was not attenuated by exogenous glucose or GSH. Figure 4B shows retention of peroxide in ileal tissue exposed to lipid hydroperoxides over 30 min. The data show that even before exposure to peroxidized lipids, ileal intestinal tissue from hypoxic rats exhibits significantly higher endogenous peroxide production compared with normoxic intestine, correlating with a previously described hypoxia-induced oxidative stress in rat small intestine (21, 22). After 30-min exposure to lipid hydroperoxides, hypoxic ileal intestine continues to exhibit significantly greater levels of peroxide retention than normoxic tissue, whether in the presence or absence of exogenously supplied glucose or GSH. Data from normoxic and hypoxic jejunum are shown in Table 1. As with the hypoxic ileum, tissue peroxide concentration in the hypoxic jejunum was significantly higher than normoxic tissue before lipid hydroperoxide exposure (control) and exhibited a trend similar to that observed in hypoxic ileum under the other conditions, although only the hydroperoxide plus glucose condition achieved statistical significance.

Effect of exposure to peroxidized lipids on mucosal thiol/disulfide status and protein thiol concentration. Figure 5A shows mucosal GSH concentration after 30-min exposure to lipid hydroperoxides. Before exposure to peroxidized lipids, GSH levels in ileal tissue were similar in normoxic and hypoxic intestine. Control GSH levels appear lower than those reported in previous studies (6), but this difference may be due to GSH efflux (25) during eversion of intestinal sacs and/or during a 20-min prewarming period before incubation with peroxidized lipids. Thus absolute GSH values may be underestimates, but comparison of trends between experimental conditions should be unaffected because all tissue segments were treated similarly. After 30-min exposure in the absence of added substrates, GSH concentration in both conditions was significantly decreased. This decrease was attenuated in normoxic intestine in the presence of exogenous glucose or GSH, but addition of substrates did not preserve GSH concentration in hypoxic tissue, corroborating our previous finding that hypoxic enterocytes lack the ability to utilize glucose to maintain constant cell GSH (22). Although GSH levels were greatly reduced by peroxidized lipid exposure, the ratio of GSH to GSSG did not change (data not shown), possibly due to efflux of GSSG from the cells under these conditions or to the interaction of GSSG with protein thiols.

An important function of cellular GSH is preservation of the reduced state of cellular protein thiols (protein-bound sulfhydryl groups), which are critical to the function of many enzymes and other proteins in the cell. Figure 5B shows that before exposure to peroxidized lipids, baseline protein thiol concentration in ileal mucosa was similar in normoxic and hypoxic intestine. After exposure to peroxidized lipids, protein thiol concentration in hypoxic intestine was significantly decreased compared with normoxic tissue and was not restored by the addition of glucose or GSH. Protein thiol concentration in normoxic intestine, on the other hand, was preserved in the presence of exogenous glucose or GSH during exposure to peroxidized lipids. Data from normoxic and hypoxic jejenum are shown in Table 2. Incubation of normoxic jejenum with lipid peroxides caused modest decreases in tissue GSH and protein thiols that were preserved by exogenous glucose and GSH. In contrast, exposure of hypoxic jejenum...
to lipid peroxides resulted in significant decreases in GSH and protein thiol levels that were not restored by glucose or GSH.

Effect of chronic hypoxia on glucose uptake by enterocytes. Because hypoxic intestinal cells appear to be unable to utilize exogenous glucose to enhance detoxication capacity, it is important to determine if glucose is gaining access to the cells. To determine glucose uptake, isolated enterocytes were incubated for 30 min with 10 mM [14C]glucose. Figure 6 shows that there is no difference in glucose uptake between normoxic and hypoxic cells, indicating that the lack of glucose dependence for peroxide elimination in hypoxic intestine is not due to decreased uptake. Glucose uptake was similar in normoxic and hypoxic jejunum (data not shown).

Diamide infusion and determination of NADPH supply rate. An alternate explanation for the inability of hypoxic enterocytes to utilize glucose for augmentation of peroxide elimination is that glucose is being diverted from the pentose phosphate pathway and therefore from production of NADPH for GSSG reduction. To test this suggestion, the cellular rate of NADPH supply was measured using an approach that was previously established for hepatocytes (32). This method utilizes controlled infusions of diamide, a thiol oxidant, into cell suspensions and subsequent quantification of the cells' ability to maintain a constant GSH level. As shown in Fig. 1, the rate of NADPH supply for hydroperoxide elimination can be determined by the rate of GSSG reduction to maintain a steady-state GSH pool within the cell. Based on this reasoning, the rate of GSH oxidation by diamide at which the cell becomes unable to maintain steady-state GSH levels is equal to the maximal NADPH supply rate. The diamide infusion rate at this “break point” is termed the critical infusion rate (3, 32). Thus determination of critical diamide infusion rates in normoxic and hypoxic enterocytes provides a reasonable estimate of the NADPH supply rate for GSH regeneration from GSSG under our experimental conditions.

In the absence of diamide, cell GSH was maintained equally well in normoxic and hypoxic cells for the 15-min experimental period (Fig. 7). Infusion of diamide into suspensions of ileal cells from normoxic rats had little effect on these cells' ability to maintain a constant GSH pool (Fig. 7A). Under hypoxic conditions, however, diamide infusion caused a dose- and time-dependent decrease in cell GSH (Fig. 7B). Constant cell GSH in hypoxic enterocytes was maintained at lower infusion rates, up to 0.07 nmol diamide·min⁻¹·10⁶ cells⁻¹, but the GSH pool progressively decreased over time and as diamide concentration increased. For example, at 15 min there was a significant decrease in the cellular GSH pool at a diamide infusion rate of 0.4

**Table 2.** GSH and protein thiol concentrations in jejunum after 30 min exposure to lipid hydroperoxides

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LH Only</th>
<th>LH + Glc</th>
<th>LH + GSH</th>
<th>LH + Glc + GSH</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GSH, nmol/g tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nx</td>
<td>129.4 ± 5.4</td>
<td>72.4 ± 2.7†</td>
<td>118.0 ± 7.1</td>
<td>106.1 ± 6.5</td>
<td>107.2 ± 6.1</td>
<td>4</td>
</tr>
<tr>
<td>Hx</td>
<td>123.7 ± 9.2</td>
<td>51.6 ± 5.4*</td>
<td>57.4 ± 4.2*</td>
<td>55.3 ± 1.4*</td>
<td>66.5 ± 4.3*</td>
<td>4</td>
</tr>
<tr>
<td><strong>Protein thiols, μmol/g tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nx</td>
<td>1.8 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>10</td>
</tr>
<tr>
<td>Hx</td>
<td>1.6 ± 0.2</td>
<td>0.7 ± 0.0*</td>
<td>0.9 ± 0.0*</td>
<td>0.9 ± 0.1*</td>
<td>0.9 ± 0.0*</td>
<td>14</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, number of animals. Hx, chronic hypoxia; Nx, pair-fed normoxic controls. †P < 0.05 vs. Nx control; *P < 0.05 vs. Hx control and Nx under same condition.
nmol·min⁻¹·10⁶ cells⁻¹, and at the highest infusion rate of 0.8 nmol·min⁻¹·10⁶ cells⁻¹ significant GSH decrease occurred at 5 min (Fig. 7B). This failure of hypoxic cells to maintain GSH with increasing diamide concentration indicates that the rate of GSH oxidation exceeded that of GSSG reduction by NADPH at these higher concentrations of the thiol oxidant.

To determine the maximal NADPH supply rate, data from Fig. 7 were replotted to show decreases in GSH (Fig. 8A) and the ratio of GSH to GSSG (Fig. 8B) as a function of the diamide infusion rate. From this relationship, we estimated the critical diamide infusion rate (the rate at which cell GSH fell), which occurred at >0.8 nmol·min⁻¹·10⁶ cells⁻¹ for normoxic enterocytes and at 0.4 nmol·min⁻¹·10⁶ cells⁻¹ for hypoxic enterocytes. Addition of 10 mM glucose to the incubation medium resulted in preservation of the cellular GSH pool at all doses of diamide in normoxic as well as hypoxic cells (Fig. 8A). Changes in the ratio of GSH to GSSG (Fig. 8B) were similar to those for GSH alone. Diamide infusion (0.4 and 0.8 nmol·min⁻¹·10⁶ cells⁻¹) caused significant oxidative stress in hypoxic enterocytes, which was abrogated by glucose addition, consistent with a greater vulnerability of the hypoxic intestine to oxidant injury. GSH data from jejunum are shown in Table 3.

Diamide infusion caused no significant decrease in GSH in normoxic enterocytes, but cell GSH was significantly decreased in hypoxic cells. The loss of cell GSH in both conditions was largely prevented by glucose addition. Changes in the jejunal GSH-to-GSSG ratio were similar to those in ileal tissue during diamide infusion at 0.8 nmol·min⁻¹·10⁶ cells⁻¹ without added glucose, causing a significant oxidative stress (normoxia, 7.1 ± 2.1 vs. 0.4 ± 0.1 in hypoxic jejunum).

**DISCUSSION**

Our previous studies have shown that GSH availability is a key determinant of metabolism and elimination of toxic hydroperoxides by the intestine (5, 6). In view of the fact that GSH uptake by the intestine is impaired by chronic hypoxia (7), a critical source of intracellular GSH for peroxide detoxication in the hypoxic intestine is from the reduction of GSSG by the GSH redox system. Our laboratory has shown that glucose availability is an important factor regulating production of the reductant NADPH for GSH regeneration by this redox system (3). Glucose flux through the pentose phosphate pathway is responsible for much of the NADPH production in enterocytes (3). As shown in Fig. 1, hydroperoxides are reduced at the expense of GSH, and regeneration of GSSG by NADPH maintains the thiol redox balance within the cell. If glucose flux is diverted from this pathway, decreased availability of...
NADPH compromises cell GSH concentration. In the current study, we have shown that enterocytes from hypoxic intestine are substrate limited by the amount of glucose available for use by the pentose phosphate pathway and NADPH production. We have shown previously that a variety of metabolic aberrations are present in the hypoxic intestine (22). For example, the initial rate of hydroperoxide metabolism by chronically hypoxic enterocytes is greatly exaggerated compared with that of normoxic cells. We also found that exogenously supplied glucose significantly increases hydroperoxide metabolism in normoxic cells but has little effect on augmentation of peroxide elimination in hypoxic cells. Wide swings in mitochondrial O2 consumption during substrate (glucose) or oxidant (tert-butyl hydroperoxide) challenge led us to conclude that hypoxic enterocytes exhibit loss of mitochondrial regulation and thus inherent metabolic instability, appearing to operate in a “hyperdynamic” state in which they continuously metabolize not optimally but maximally. It has been reported (12) that patients with an unresolved focus of stress exhibit a prolonged hypermetabolic response, predisposing them to development of progressive metabolic dysregulation. Thus prolonged O2 deficiency appears to impose an ongoing stress on tissues, similar to that accompanying burn, trauma, or sepsis, all of which are associated with the development of a hypermetabolic state (30), whereby metabolism may be pushed to increasingly higher levels by a systemic insult. Our current findings suggest that continually elevated metabolism in the intestine could lead to failure of intestinal function in the face of a secondary challenge, such as exposure to luminal lipid hydroperoxides.

In this study peroxide transport into the contraluminal compartment of everted intestinal sacs represents transport of peroxides into lymph, where they gain access to the systemic circulation. Both jejunum and ileum were used in our experiments, because functions such as nutrient absorption and active transport of bile salts are often specific to a given segment of intestine. Thus differences in experimental results between segments would be expected to impact intestinal function differently. Our data demonstrate that initial lipid hydroperoxide transport into the contraluminal compartment by hypoxic and normoxic intestine is low, suggesting that the hypoxic intestine can handle peroxide elimination during short-term peroxide exposure. However, the detoxication capacity in hypoxic intestine is decreased with continual peroxide stress, as evidenced by an increase in contraluminal transport of hydroperoxides. This finding is consistent with our

Table 3. GSH concentration in jejunum during diamide infusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.4 nmol·min⁻¹·10⁶ cells⁻¹</th>
<th>0.8 nmol·min⁻¹·10⁶ cells⁻¹</th>
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<tr>
<td></td>
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<tr>
<td>GSH in absence of glucose, nmol/10⁶ cells</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nx (n = 6)</td>
<td></td>
<td></td>
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<tr>
<td>0 min</td>
<td>2.1 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>1.6 ± 0.4</td>
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<tr>
<td>5 min</td>
<td>1.7 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>15 min</td>
<td>1.3 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.2</td>
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<tr>
<td>Hx (n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>2.1 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>5 min</td>
<td>2.0 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>0.6 ± 0.2*</td>
</tr>
<tr>
<td>15 min</td>
<td>1.4 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.2 ± 0.1*</td>
</tr>
<tr>
<td>GSH in presence of glucose, nmol/10⁶ cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nx (n = 3)</td>
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<td></td>
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<tr>
<td>0 min</td>
<td>3.3 ± 0.0</td>
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<tr>
<td>5 min</td>
<td>2.7 ± 0.1</td>
<td>2.6 ± 0.2</td>
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<tr>
<td>15 min</td>
<td>2.2 ± 0.3</td>
<td>2.0 ± 0.1</td>
<td>1.4 ± 0.2</td>
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<td>Hx (n = 4)</td>
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<tr>
<td>0 min</td>
<td>3.5 ± 0.6</td>
<td>3.2 ± 0.6</td>
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<tr>
<td>5 min</td>
<td>2.8 ± 0.5</td>
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<tr>
<td>15 min</td>
<td>2.3 ± 0.6</td>
<td>1.8 ± 0.5</td>
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Data are means ± SE; n, number of animals. Control, no diamide. *P < 0.05 vs. its own control at same time point.
hypothesis depicted in Fig. 2 and previous studies (5, 6), wherein increased transport into lymph is consistent with impaired hydroperoxide metabolism by the GSH redox system in the cell. The data are also consistent with our contention that the GSH-deficient state is the predominant one under hypoxic conditions. This suggestion is supported by the finding that endogenous hydroperoxide production in intestinal tissue before hydroperoxide exposure is significantly higher in the hypoxic state. These results corroborate our earlier study in which the hypoxic state was associated with elevation of TBARS in the urine of rats and a compromised thiol-to-disulfide ratio in the intestine (21).

Our current data show that contraluminal transport of hydroperoxides by normoxic, but not hypoxic, enterocytes is attenuated by exogenous GSH or glucose, consistent with decreased utilization of GSH and glucose to support GSH redox function in peroxide metabolism in hypoxic intestine. This finding is not surprising in view of the fact that GSH uptake is impaired in the hypoxic intestine (7), and previous studies in our laboratory have shown that glucose supplementation does not enhance hydroperoxide elimination in hypoxic cells (22). Importantly, the enhanced oxidative stress consequent to decreased peroxide detoxication in the hypoxic intestine causes significant oxidation of protein thiols. Because preservation of thiols is critical to the function of cellular enzymes and other proteins, substantial protein thiol oxidation would have important consequences for cell integrity. Moreover, the added inability of the hypoxic intestine to restore cell GSH and redox homeostasis with exogenous glucose and GSH suggests that this organ is highly susceptible to oxidant-induced injury. These findings strongly support our contention that hypoxic intestine, already in a state of oxidative stress, is incapable of maintaining its functional integrity in the face of additional peroxide challenge (21, 22).

Previous studies have shown that both acute (23) and chronic hypoxia (7) were associated with decreased intestinal nutrient absorption. It is interesting that in our study we found that the hypoxic intestine is just capable of glucose uptake as the normoxic intestine. Thus the inability of glucose to stimulate peroxide detoxication in the hypoxic intestine cannot be explained on the basis of decreased glucose uptake by enterocytes. Rather, our data are consistent with a reduction of glucose flux through the pentose phosphate shunt to support NADPH production for GSSG reduction. Our previous studies have shown that exogenous glucose significantly increases mitochondrial respiration (22). These results suggest that both endogenous and exogenous glucose may preferentially be diverted to support primary metabolic demands, e.g., mitochondrial function, in the hypoxic intestine, thereby decreasing glucose utilization by secondary metabolic pathways such as the pentose phosphate pathway to support peroxide detoxication. Consequently, the hyperdynamic metabolic state associated with chronic hypoxia leaves the hypoxic intestine with little critical reserve to deal with additional challenges, such as the presence of luminal lipid hydroperoxides. The compromised state of the hypoxic intestine could result in local damage to the mucosa, as well as in enhanced transport of peroxidized lipid into the circulation via lymph. These oxidant-induced changes have important implications for the genesis of gut pathologies and atherosclerosis, respectively.

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