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Determinants of Intestinal Detoxication of Lipid Hydroperoxides

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It has long been recognized that hydroperoxides are agents of cytotoxicity. However, in recent years, it is increasingly apparent that lipid hydroperoxide may play an important role in mediating cellular and molecular events in degenerative pathophysiological processes that lead to intestinal disorders, such as cancer. Yet, surprisingly, little is known of the intestinal disposition of peroxidized lipids and of the metabolic factors that determine mucosal peroxide elimination. The present paper summarizes the evidence for the pivotal role of reductant (GSH and NADPH) availability in intestinal peroxide detoxication. This information will provide important insights into the relationship between luminal lipid hydroperoxides and intestinal GSH redox homeostasis, and is pertinent to understanding how dietary oxidants like lipid peroxides, can impact intestinal integrity with implications for genesis of gut pathology.

Keywords: GSH, luminal lipid hydroperoxides, intestinal GSH redox cycle, lymph and bile fistula rat, intestinal hydroperoxide absorption and transport, intestinal glucose supply, NADPH and pentose phosphate shunt, intestinal peroxide detoxication, mucosal redox balance

1. INTRODUCTION

Dietary intake of highly polyunsaturated fats represents a major source of lipid hydroperoxides in the intestinal lumen. Under conditions of high peroxide intake, excessive concentrations of lipid hydroperoxides can persist in the gut lumen and contribute to impairment of mucosal detoxication pathways, enterocyte dysfunction independent of cell injury, and development of gut pathologies, such as cancer. For many years, thiols like glutathione (GSH) have been known to have critical functions in the gastrointestinal tract, and there has been much interest in the role of GSH in protection against dietary oxidants and endogenously produced reactive oxygen species. This paper summarizes our current knowledge of the role of GSH in intestinal lipid hydroperoxide metabolism and of the physiological determinants of lipid peroxide detoxication in the small intestine. The discussion is pertinent to understanding dietary lipid peroxides and GSH redox balance in

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intestinal physiology and pathophysiology and the significance of luminal GSH in preserving metabolic integrity of the intestinal epithelium.

2. PEROXIDIZED LIPIDS IN INTESTINAL PATHOLOGY

Peroxidized lipids represent a class of dietary oxidants of nutritional and toxicological importance, and are among a variety of natural mutagens and carcinogens present in the human diet which can initiate degenerative processes via generation of oxygen radicals. There is growing recognition that radical-mediated degenerative processes can lead to disorders of the digestive system,[1,2] including intestinal inflammation and cancer[3] that are major health problems in humans.

Lipid hydroperoxides are toxic products of peroxidized polyunsaturated fatty acids of membrane lipids and dietary fats.[4,5] Previous studies by several investigators have shown a causal relationship between the toxicity of dietary polyunsaturated oils with their peroxide content,[6–8] thus providing evidence for cytotoxicity associated with excessive consumption of lipid hydroperoxides *in vivo*. While human consumption of lipid hydroperoxides is unlikely to achieve cytotoxic levels, high intake of dietary polyunsaturated fatty acids can contribute to the luminal accumulation of lipid hydroperoxides. Subsequent lipid peroxidation and oxyradical generation can induce tissue oxidative stress and redox imbalance with significant impact on intestinal integrity. Early findings have implicated dietary fat as a risk factor for development of gut malignant transformation in humans, and high intake of dietary fats has been linked to increased incidence of colon cancer.[9–11] What was not recognized then is the fact that substantial intake of lipid hydroperoxides would accompany the consumption of highly unsaturated fats with potential deleterious effect on gastrointestinal homeostasis. Indeed, enhancement of tumorigenesis in the colon has been shown to be associated with local administration of oxygenated derivatives of unsaturated fatty acids.[12–14] Bull *et al.* found that instillation of hydroperoxy and hydroxy fatty acids intrarectally provoked proliferative responses in colonic mucosa in rats that correlated with stimulation of DNA synthesis and induction of ornithine decarboxylase.[12] Moreover, Hara *et al.* found that rats given oxidized ethyl linoleate (peroxide value of 1400 mEq/kg lipid) developed mucosal hypertrophy of the large intestine.[15] Collectively, these findings demonstrate that normal intestinal cell turnover can be disrupted by lipid hydroperoxides and thus, underscore the tumorigenic potential of oxidized lipids.

Despite this recognition, and the implication of dietary peroxidized lipids in gut pathologies, we know little of the underlying mechanisms in the development of the disease processes or of the pathways of intestinal detoxication and luminal disposition of dietary lipid hydroperoxides *in vivo*.

Recent studies from our laboratory has provided new insights in that hydroperoxide detoxication in the intestine is far more dependent on reductant (GSH and NADPH) supply than other major organs of detoxication such as the liver. The following sections will review the evidence supporting the contention that overall mucosal detoxication of toxic lipid hydroperoxides largely subscribes to regulation by GSH and NADPH availability.

3. GSH REDOX CYCLE IN INTESTINAL HYDROPEROXIDE DETOXICATION

GSH is the cellular reductant for GSH peroxidase (GSH Px) catalyzed elimination of a variety of hydroperoxides, including H_2O_2, organic hydroperoxides and lipid hydroperoxides.[16,17] Typically, during hydroperoxide reduction, GSH is oxidized to GSSG, and is regenerated by GSSG reductase (GSSG Rd) at the expense of cellular reduced nicotinamide adenine dinucleotide phosphate (NADPH).[16,17] Thus, the GSH redox

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system provides a general mechanism for hydroperoxide catabolism in tissues, including the intestine. The efficiency of function of this redox cycle in peroxide catabolism will depend on the optimal activities of the redox enzymes, GSH Px and GSSG Rd, and the optimal supply of the reductant GSH.

Studies from our laboratory and others indicate that intestinal GSH Px and GSSG reductase functions are minimally modified under different physiological and pathophysiological states such as chronic O_2 deficiency,[18] diabetes[19] and peroxide challenge.[17,20] Hence, it appears that regulation of enzyme expression play a relatively minor role in the overall detoxication of lipid hydroperoxides in the intestine. It is notable, however, that a unique intestinal isoform of GSH Px has been described, designated GSH PX-GI,[21] suggesting a potential role for this enzyme in intestinal detoxication reactions. A physiological function has not been ascribed for this newly described intestinal GSH PX-GI, but the enzyme appears to function like the classical GSH Px in that it catalyzes reduction of H_2O_2 and *tert*-butyl hydroperoxide.[21] However, at a catalytic rate of one-tenth of that of GSH Px, the contribution of GSH PX-GI to the quantitative metabolism of lipid hydroperoxides in the intestine cannot be fully appreciated at present.

Although metabolism of lipid hydroperoxides in the intestine does not appear to be rate-limited by redox enzyme activities, recent evidence suggests that reductant availability is a major determinant of this redox detoxication pathway. Indeed, substrate availability is one of several key cellular regulatory mechanisms in the control of metabolic pathways.

4. GSH AVAILABILITY FOR INTESTINAL LIPID HYDROPEROXIDE METABOLISM

Glutathione (GSH) is one of several naturally occurring antioxidants, such as -tocopherol (vitamin E), -carotene (vitamin A), and ascorbic acid (vitamin C), as well as cellular antioxidant enzymes, such as superoxide dismutase, catalase and GSH peroxidase that function to detoxify reactive oxygen metabolites of endogenous or exogenous origins. GSH is a ubiquitous tripeptide (-glu-cys-gly) that is present in high concentrations in tissues.[22,23] Normal cellular GSH homeostasis is maintained by *de novo* synthesis from sulfur-containing precursor amino acids and regeneration from glutathione disulfide, GSSG.[22,23] In certain cell types, cellular GSH concentrations can also be enhanced by exogenous GSH uptake via Na^+-dependent transport systems, such as enterocytes,[24] type II alveolar cells,[25] renal proximal tubular cells,[26] and endothelial cells.[27] In these cells, the uptake of exogenous GSH afforded protection against peroxide-induced cell damage, thus demonstrating the importance of exogenous GSH as a source of reductant for promoting intracellular peroxide metabolism.

Luminal GSH in Peroxide Elimination

GSH posseses two important structural features crucial to its biological function: a reactive thiol (SH) group and a -glutamyl bond that is resistant to proteolytic cleavage.[22] Several animal studies have demonstrated the uptake of intact GSH from the luminal surface of the small intestine *in vivo* to supplement cellular GSH pools[28,29] which supports the idea that luminal uptake of GSH play a part in maintaining mucosal GSH status that is crucial for normal intestinal function, including detoxication reactions. Martensson *et al.* found that oral administration of GSH prevented severe degeneration of jejunal and colonic epithelial cells induced by chronic GSH deficiency[30] which lends further support for a role of luminal GSH in the maintenance of the functional integrity of the intestinal epithelium.

The major sources of luminal GSH are from the diet[31] and from biliary output in bile.[22] To date, the physiological role for luminal GSH in intestinal detoxication reactions is unresolved.

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However, in recent studies, we have provided evidence that luminally derived GSH is an important source of reductant for intracellular catabolism of lipid hydroperoxides.[32–34] In these studies, we established a conscious lymph fistula rat model to quantify the intestinal disposition of luminal lipid hydroperoxides *in vivo*.[32,33] In this rat model, peroxidized lipid is infused intraduodenally and steady-state lymph output is monitored via the major mesenteric lymph duct. As illustrated in the working model in Figure 1A, the amount of peroxide recovered in the intestinal lumen and intestinal lymph is dependent on the mucosal GSH status at a given dose of infused lipid hydroperoxide (represented by LOOH in Figure lA). Under GSH sufficient conditions, increased peroxide metabolism within the intestinal mucosa drives luminal LOOH absorption (resulting in low luminal retention) and decreases peroxide output into lymph (upper panel, Figure 1A). Conversely, under GSH deficient states, a decrease in intracellular peroxide catabolism results in reduced luminal LOOH absorption (hence, high luminal LOOH retention) as well as enhanced LOOH transport into lymph (lower panel, Figure 1A). Thus, it appears that the major driving force for elimination of lipid hydroperoxides from the intestinal lumen is largely derived from the metabolic capacity of the enterocytes to catabolize peroxides intracellularly. This mechanism for peroxide removal from the gut lumen is analogous to regulation of biochemical reactions by metabolic trapping and offers an efficient mode for rapid elimination of peroxidized lipids by the small intestine.

Experimentally, simultaneous quantification of hydroperoxide recovered in intestinal lumen and intestinal lymph would provide reasonable estimates of the intestinal capacity for lipid hydroperoxide detoxication *in vivo*. Using the lymph fistula rat model, we have documented that the intestinal absorption and lymphatic transport of

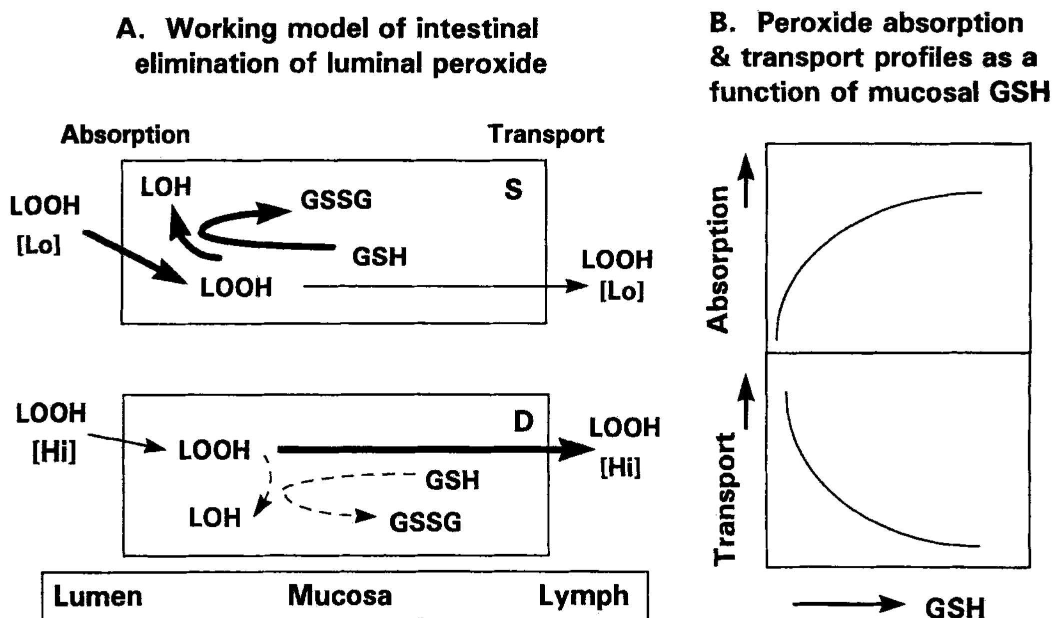

FIGURE 1 (A) Working model for intestinal lipid hydroperoxide absorption and lymphatic transport under conditions of GSH sufficiency and GSH deficiency. The abbreviations are: GSH, GSSG: reduced and oxidized glutathione respectively; S, D: GSH sufficiency and deficiency, respectively; LOOH, LOH: lipid hydroperoxide and hydroxide, respectively; Lo, Hi: low and high peroxide concentrations, respectively. (B) Intestinal profiles of lipid hydroperoxide absorption and lymphatic transport as a function of mucosal GSH concentrations. Increases in mucosal GSH enhance intestinal peroxide metabolism intracellularly and thus, drive peroxide uptake from gut lumen and reduce peroxide output into lymph.

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lipid hydroperoxides *in vivo* is a function of the mucosal GSH content (Figure 1B).[32] We found that, by lowering cellular GSH, the recovery of the infused lipid hydroperoxide was enhanced as was the steady-state output of lipid peroxides into lymph (Figure 1B),[32] consistent with a decreased intracellular metabolism under GSH-deficient conditions. Thus, maintenance of mucosal GSH status is important for the quantitative removal of luminal peroxidized lipids by the small intestine. Furthermore, supplementation of GSH-deficient lymph fistula rats with physiological concentrations of GSH (between 100 μM–1 mM) dose-dependently increases mucosal GSH and decreases luminal and lymph peroxide contents.[33] This shows that mucosal GSH levels can be effectively restored by exogenous GSH supplementation to support intestinal metabolism of luminal peroxidized lipids and reduce peroxide transport into lymph, consistent with previous observations in isolated intestinal everted sacs.[35] Importantly, these findings underscore the fact that exogenous luminal GSH can serve as a quantitative source of reductant for mucosal metabolism of dietary peroxidized lipids during an acute exposure of the intestine to a physiological lipid peroxide load.

In the intestine, GSH is also made available to the mucosa by way of hepatic export of GSH into bile. Kaplowitz *et al.* reported that GSH output into bile is directly proportional to the GSH concentration in liver.[22] It is estimated that at least 50–60% of hepatic GSH output goes into bile,[22] thus biliary GSH can achieve millimolar concentrations (2–10 mM). We recently demonstrated the importance of biliary GSH in intestinal peroxide detoxication in a lymph and bile-fistula rat model. In these animals, the bile duct was cannulated to divert biliary GSH. Luminal and lymph recoveries of lipid hydroperoxides were substantially higher than controls.[34] Using the same analysis and reasoning based on the proposed model in Figure 1A these results are consistent with decreased intracellular hydroperoxide metabolism in the intestine of bile fistula animals. Additional studies show that exogenous GSH supplementation restored the intestinal capacity to metabolize luminal peroxides in these bile-diverted animals,[34] indicating that GSH is the predominant component in bile that determines peroxide elimination by the small intestine. To what extent biliary GSH contributes quantitatively to enterocyte GSH homeostasis under physiological and pathophysiological state, as compared to GSH synthesis and GSSG reduction, is not known. Also unknown is the extent to which luminal GSH preserves intestinal integrity with prolonged exposure of the gut to lipid hydroperoxides. However, given that the human diet varies considerably in GSH levels[31] and in lipid hydroperoxide content[36,37] and that luminal GSH varies depending on biliary output, the above considerations will have important toxicological and nutritional implications.

5. NADPH AVAILABILITY FOR PEROXIDE DETOXICATION

The maintenance of GSH availability to support function of the GSH redox cycle ultimately depends on the supply of NADPH and is functionally coupled to the pentose phosphate pathway (Figure 2). Typically, increased cellular GSSG results in activation of NADPH supply to support GSSG reduction to maintain homeostasis.[38] While cellular NADPH homeostasis can be maintained by several intracellular sources, namely, mitochondrial NADH transhydrogenation[39] and $NADP^+$ specific dehydrogenases,[23] the major contributor to the total cellular NADPH pool is the pentose phosphate pathway.[40] This shunt is regulated by glucose 6-phosphate dehydrogenase activity and the supply of glucose.[41] Given the relatively slow GSH synthetic rate in enterocytes[42] as compared to hepatocytes,[43] GSSG reduction may represent the major pathway for maintaining constant cell GSH in the intestine under high rates of lipid hydroperoxide metabolism. Consequently, an enhanced demand

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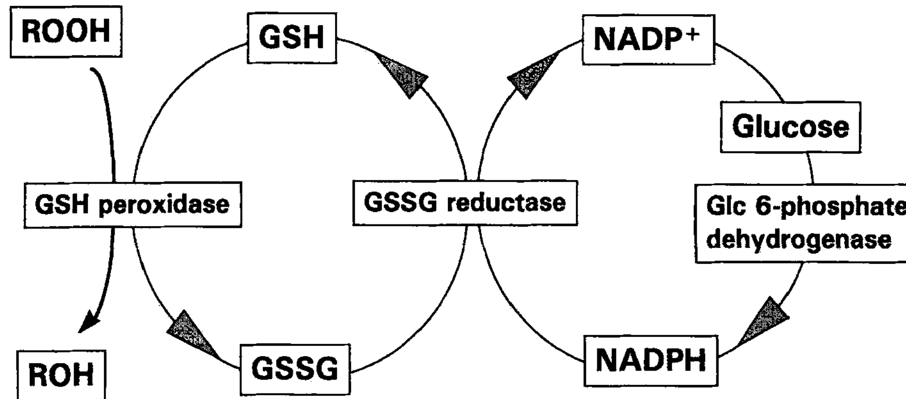

FIGURE 2 Coupling of pentose phosphate shunt to GSH redox cycle function in hydroperoxide detoxication.

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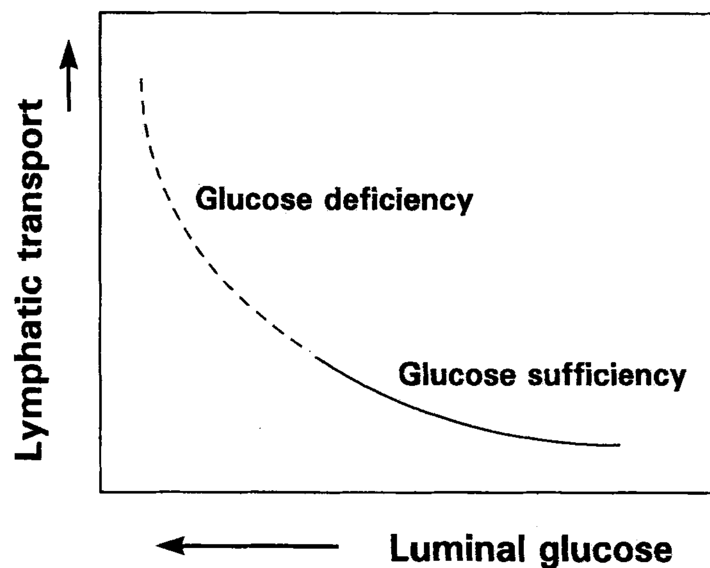

FIGURE 3 Lymphatic transport of lipid hydroperoxides under glucose sufficient and deficient conditions. Insufficiency of luminal glucose compromises intestinal detoxication of lipid hydroperoxides resulting in increased peroxide transport into intestinal lymph. (——), luminal glucose sufficiency; (----), luminal glucose deficiency.

for GSSG reduction due to exaggerated GSH oxidation could exceed the availability of NADPH.[44] The resultant extensive depletion of the GSH pool will result in cessation of further peroxide degradation and compromise intestinal cell integrity.

In liver cells, metabolism of organic hydroperoxides like *tert*-butyl hydroperoxide (*t*BH), is efficient and appears to be independent of exogenous glucose supply. In contrast, we found that in intestinal cells, *t*BH is highly sensitive to the availability of glucose. The endogenous rate of *t*BH metabolism in enterocytes is low,[44] about one-fifth the rate in hepatocytes,[45] but increased markedly with exogenous glucose.[44] That *t*BH metabolism in enterocytes from 24 h fasted rats occurred at only one-third the rate of cells from fed animals[44] lends further support for a reliance on glucose availability for efficient elimination of *t*BH. Additional studies reveal that

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mechanistically, the glucose effect is mediated through stimulation of the pentose phosphate shunt activity to increase NADPH supply for GSSG reduction.[44]

In other studies in the lymph fistula rat, the removal of glucose in the lipid peroxide infusate in GSH-sufficient animals resulted in elevated luminal and lymph hydroperoxides (Figure 3), profiles that are similar to those of the GSH-deficient state (Figure 1B).[32,33] One reasonable explanation for these latter results is that despite an initial high GSH level, this pool, if not regenerated to keep pace with constant peroxide challenge, can readily be depleted. Thus, to sustain hydroperoxide detoxication at constant cell GSH, glucose supply is critical to maintain steady-state production of NADPH by the pentose phosphate shunt to support continued function of the GSH redox cycle. Taken together, these findings suggest that mucosal detoxication of hydroperoxides would subscribe to regulation by the luminal and/or plasma glucose concentration. Furthermore, the difference in reliance on glucose between liver and intestines suggest organ-specific regulation of hydroperoxide detoxication in different tissues.

Quantification of NADPH Availability for GSSG Reduction in Enterocytes

Tribble and Jones have previously developed and characterized the diamide-induced GSH redox for estimating NADPH supply rate in isolated hepatocytes.[45] This approach relies on the simple principle that, in the presence of a steady-state oxidant load, such as hydroperoxide challenge, constant cell GSH levels can be maintained if the rate of GSH oxidation by the oxidant is matched by the rate of GSSG reduction by NADPH. The thiol oxidant, diamide, offers a suitable agent to quantify the rate of NADPH supply since diamide preferentially reacts with cellular GSH to produce GSSG at constant rates;[44,45] thus, the rate at which GSSG is reduced will be a measure of the rate of supply

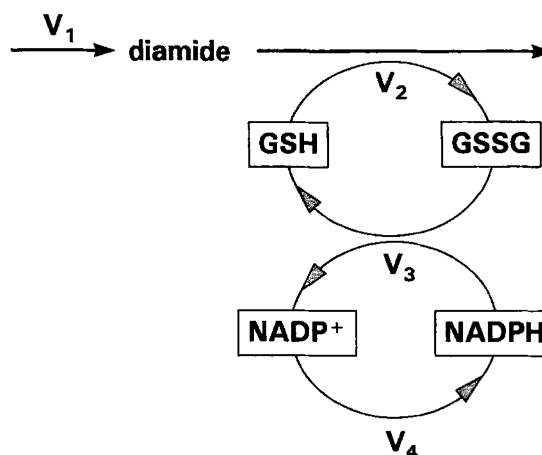

FIGURE 4 The diamide-induced GSH redox cycle model for quantification of NADPH supply rate in isolated enterocytes. V_1, V_2, V_3, and V_4, represent the respective rates of diamide infusion, GSH oxidation, GSSG reduction, and NADPH supply. Redrawn from Tribble and Jones.[45]

of NADPH.[44] This model is conceptually depicted in Figure 4. A constant and known rate of GSSG generation (V_2) is achieved using steady-state rates of infusion of diamide (V_1). The rate of GSSG reduction (V_3) under steady-state conditions will equal the rate of diamide infusion such that V_1 will also equal the total NADPH supply for GSSG reduction from all sources within the enterocyte (V_4). The diamide infusion rate can be increased to obtain conditions in which the cell is unable to maintain a steady-state; at this "break-point", the rate of diamide infusion will be termed the critical infusion rate.[44,45] At this point where GSH oxidation exceeds GSSG reduction, maximal supply of NADPH is reached. Experimentally, this critical point is achieved when cell GSH falls in response to an oxidant like hydroperoxide. The model can readily be adapted to assess the quantitative contribution of the pentose phosphate shunt to the total cellular supply of NADPH by pretreating cells with 6-aminonicotinamide, an inhibitor of glucose 6-phosphate dehydrogenase.[40,41]

Previous estimates of NADPH supply in hepatocytes were in the range of 5–8 nmol min^{-1} per 10^6 cells.[39,45–48] In enterocytes, we found that

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the maximal NADPH supply rate in the absence of glucose is 100-fold lower than that in liver cells ($0.05\,\text{nmol}\,\text{min}^{-1}$ per 10^6 cells),[44] indicating a substantially lower capacity for hydroperoxide detoxication in the intestine as compared to the liver. Moreover, with exogenous glucose, the maximal intestinal reductant supply rate increased 10-fold (0.5–$1.0\,\text{nmol}\,\text{min}^{-1}$ per 10^6 cells),[44] but is still 5–10 times lower than that in liver.[45] In more recent studies, we found that the ability of enterocytes to detoxify *t*BH and to utilize glucose for NADPH production are markedly decreased when animals were subjected to chronic hypoxia (10 days at 10% O_2)[49] or placed chronically on a subtoxic peroxidized lipid diet (4–8 weeks at 2–10 μmol peroxides/20 g chow per day).[50] Collectively, these results suggest a compromised capacity of the small intestine for handling high concentrations of hydroperoxides. This deficiency can be exacerbated under conditions of decreased nutrient availability, such as occurs during fasting or under pathophysiological diseased states, such as during chronic hypoxia[18,49,51] as occurs in chronic obstructive pulmonary disease, cystic fibrosis, and cardiovascular insufficiency.[52]

6. CONCLUDING REMARKS

Despite our extensive knowledge on the chemistry of lipid peroxidation and the deleterious role that this process plays in cytotoxicity in a variety of tissues, the current understanding of the physiological determinants of disposition of luminal peroxidized lipids and the influence of luminal peroxides in intestinal thiol–disulfide balance is limited. Our studies using a conscious, lymph fistula rat model have provided important insights into understanding the acute effects of peroxidized lipids on intestinal physiology *in vivo*. Significantly, this experimental approach has provided quantitative and mechanistic definition of the role of mucosal and luminal GSH in and the contribution of NADPH to intestinal intracellular peroxide elimination. This information is pertinent for understanding oxidant-mediated intestinal pathophysiology because intake of highly unsaturated fats will contribute to the accumulation of lipid peroxides in the intestinal lumen and because physiologically relevant concentrations of lipid hydroperoxides can compromise enterocyte redox homeostasis. The potential deleterious impact of redox imbalance on mucosal function and cell turnover events could have far-reaching implications with regard to development of chronic disorders of the intestine. Furthermore, the fact that the intestinal epithelium can draw upon a luminal GSH pool to promote intracellular detoxication reactions and preserve intestinal integrity should pose interesting possibilities in consideration of development of therapeutic strategies.

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References

[1] D. A. Parks, G. B. Bulkley and D. N. Granger (1983). Role of oxygen-derived free radical in digestive tract diseases. *Surgery*, **94**, 415–422.
[2] M. B. Grisham and D. N. Granger (1988). Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. *Digestive Diseases & Science*, **33**, 6S–15S.
[3] B. Ames (1983). Dietary carcinogens and anticarcinogens. *Science*, **221**, 1256–1263.
[4] A. Sevanian and P. Hochstein (1985). Mechanisms and consequences of lipid peroxidation in biological systems. *Annual Review of Nutrition*, **5**, 365–390.
[5] A. W. Girotti (1985). Mechanisms of lipid peroxidation. *Journal of Free Radical Biology and Medicine*, **1**, 87–95.
[6] J. S. Andrews, W. H. Griffith, J. F. Mead and R. A. Stein (1960). Toxicity of air-oxidized soybean oil. *Journal of Nutrition*, **70**, 199–210.
[7] T. Kaneda, H. Sakai and S. Ishii (1955). Nutritive value or toxicity of highly unsaturated fatty acids. *Journal of Biochemislry*, **42**, 561–573.
[8] T. Kimura, K. Iida and Y. Takei (1984). Mechanisms of adverse effect of air-oxidized soybean oil-feeding in rats. *Journal of Nutrition and Science Vitaminology*, **30**, 125–133.
[9] K. K. Carroll and H. T. Khor (1975). Dietary fat in relation to tumorigenesis. *Progress in Biochemical Pharmacology*, **10**, 308–353.

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[10] B. S. Reddy (1983). Dietary fat and colon cancer. In: *Experimental Colon Carcinogenesis* (eds. H. Antrup and G. M. Williams) CRC Press, Boca Raton, pp. 225–239.

[11] P. Correa, J. P. Strong, W. D. Johnson, P. Pizzolato and W. Haenszel (1982). Atheroscelrosis and polyps of the colon. Quantification of precursors of coronary heart disease and colon cancer. *Journal of Chronic Diseases*, **35**, 313–320.

[12] A. W. Bull, N. D. Nigro, W. A. Golembieski, J. D. Crissman and L. J. Marnett (1984). *In vivo* stimulation of DNA synthesis and induction of ornithine decarboxylase in rat colon by fatty acid hydroperoxides autoxidation products of unsaturated fatty acids. *Cancer Research*, **44**, 4924–4928.

[13] S. M. Fischer, G. L. Gleason, G. D. Mills and J. J. Slaga (1980). Indomethacin enhancement of TPA tumor promotion in mice. *Cancer Letter*, **10**, 343–350.

[14] A. K. Verma, C. L. Ashenden and R. K. Boutwell (1980). Inhibition of prostaglandin synthesis inhibitors of the induction of epidermal ornithine decarboxylase activity, the accumulation of prostaglandin, and tumor promotion caused by 12-O-tetradecanoylphorbol-13-acetate. *Cancer Research*, **140**, 308–315.

[15] H. Hara, K. Miyashita, S. Ito and T. Kasai (1996). Oxidized ethyl linoleate induces mucosal hypertrophy of the large intestine and affects cecal fermentation of dietary fiber in rats. *Journal of Nutrition*, **126**, 800–806.

[16] B. Chance, H. Sies and A. Boveris (1979). Hydroperoxide metabolism in mammalian organs. *Physiological Review*, **59**, 5227–5605.

[17] K. Reddy and A. L. Tappel (1974). Effect of dietary selenium and autoxidized lipids on the glutathione peroxidase system of gastrointestinal tract and other tissues in the rat. *Journal of Nutrition*, **104**, 1069–1078.

[18] T. S. LeGrand and T. Y. Aw (1996). Effect of chronic hypoxia on GSH-dependent detoxication capacity in rat small intestine. *American Journal of Physiology*, **270**, G725–G729.

[19] R. Iwakiri, C. A. Rhoads and T. Y. Aw (1995). Determinations of hydroperoxide detoxication in diabetic rat intestine: Effect of insulin and fasting on the glutathione redox cycle. *Metabolism*, **44**, 1–6.

[20] N. N. Vilas, R. R. Bell and H. H. Draper (1976). Influence of dietary peroxides, selenium, and vitamin E on glutathione peroxidase of the gastrointestinal tract. *Journal of Nutrition*, **106**, 589–596.

[21] F. F. Chu, J. H. Doroshow and R. S. Esworthy (1993). Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPX-GI. *Journal of Biological Chemistry*, **268**, 2571–2576.

[22] N. Kaplowitz, T. Y. Aw and M. Ookhtens (1985). The regulation of hepatic glutathione. *Annual Review of Pharmacology and Toxicology*, **25**, 715–744.

[23] F. C. Kauffman, R. K. Evans and R. G. Thurman (1977). Alterations in nicotinamide and adenine nucleotide systems during mixed-function oxidation of *p*-nitroanisole in rats. *Biochemical Journal*, **166**, 583–592.

[24] L. H. Lash, T. M. Hagen and D. P. Jones (1986). Exogenous glutathione protects intestinal epithelial cells from oxidative injury. *Proceedings of National Academy of Sciences USA*, **83**, 4641–4645.

[25] T. M. Hagen, L. A. Brown and D. P. Jones (1986). Protection against paraquat induced injury by exogenous GSH in pulmonary alveolar type II cells. *Biochemical Pharmacology*, **35**, 4537–4542.

[26] T. M. Hagen, T. Y. Aw and D. P. Jones (1988). Glutathione uptake and protection against oxidative injury in isolated kidney cells. *Kidney International*, **34**, 74–81.

[27] S. P. Andreoli, C. P. Mallett and J. M. Bergstein (1986). Role of glutathione in protecting endothelial cells against hydrogen peroxide oxidant injury. *Journal of Laboratory Clinical Medicine*, **108**, 190–198.

[28] T. M. Hagen and D. P. Jones (1987). Transepithelial transport of glutathione in vascularly perfused small intestine of rat. *American Journal of Physiology*, **252**, G607–G613.

[29] T. M. Hagen, G. T. Wierzbicka, B. B. Bowman, T. Y. Aw and D. P. Jones (1990). Fate of dietary glutathione: disposition in the gastrointestinal tract. *American Journal of Physiology*, **259**, G530–G535.

[30] J. Martensson, A. Jain and A. Meister (1990). Glutathione is required for intestinal function. *Proceedings of National Academy of Sciences USA*, **87**, 1715–1719.

[31] D. P. Jones, R. J. Coates, E. W. Flagg, J. W. Eley, G. Block, R. S. Greenberg, E. W. Gunter and B. Jackson (1992) Glutathione in foods listed in the National Cancer Institute's Health Habits and History Food Frequency Questionnaire. *Nutrition and Cancer*, **17**, 57–75.

[32] T. Y. Aw, M. W. Williams and L. Gray (1992). Absorption and lymphatic transport of peroxidized lipids by rat small intestine *in vivo*: Role of mucosal glutathione. *American Journal of Physiology*, **262**, G99–G106.

[33] T. Y. Aw and M. W. Williams (1992). Effect of glutathione supplementation on absorption and lymphatic transport of peroxidized lipids by rat small intestine *in vivo*. *American Journal of Physiology*, **263**, G665–G672.

[34] T. Y. Aw (1994). Biliary glutathione promotes the mucosal metabolism of luminal peroxidized lipids by rat small intestine *in vivo*. *Journal of Clinical Investigation*, **94**, 1218–1225.

[35] D. P. Kowalski, R. M. Feeley and D. P. Jones (1990). Use of exogenous glutathione for metabolism of peroxidized methyl linoleate in rat small intestine. *Journal of Nutrition*, **120**, 1115–1121.

[36] E. N. Frankel, L. M. Smith, C. L. Hamblin, R. K. Creveling and A. J. Clifford (1984). Occurrence of cyclic fatty acid monomers in frying oils used for fast foods. *Journal of American Oil and Chemical Society*, **61**, 87–90.

[37] J. C. Alexander (1986). Heated and oxidized fats. *Progress in Biological Research*, **222**, 185–209.

[38] R. Brigelius (1985). Mixed disulfides: Biological functions and increase in oxidative stress. In: *Oxidative Stress* (ed. H. Sies). Academic Press, New York. pp. 243–272.

[39] K. Weigl and H. Sies (1977). Drug oxidations dependent on cytochrome P-450 in isolated hepatocytes. *European Journal of Biochemistry*, **77**, 401–408.

[40] L. V. Eggleston and H. A. Krebs (1974). Regulation of the pentose phosphate cycle. *Biochemical Journal*, **138**, 425–435.

[41] A. Bousignone and A. De Flora (1972). Regulatory properties of glucose 6-phosphate dehydrogenase. *Current Topic in Cell Regulation*, **6**, 21–62.

[42] A. D. Williams and T. Y. Aw (1994). Glutathione (GSH) supply for lipid hydroperoxide metabolism in intestinal epithelial cells. *Gastroenterology*, **106**(4), 1054.

[43] X. Shan, T. Y. Aw and D. P. Jones (1990). Glutathione-dependent protection against oxidative injury. *Pharmacology Therapeutics*, **47**, 61–71.

[44] T. Y. Aw and C. A. Rhoads (1994). Glucose stimulation of hydroperoxide metabolism by rat enterocytes: A role

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in NADPH supply. *Journal of Clinical Investigation*, **94**, 2426–2434.
[45] D. L. Tribble and D. P. Jones (1990). Oxygen dependence of oxidative stress: rate of NADPH supply for maintaining the GSH pool during hypoxia. *Biochemical Pharmacology*, **39**, 729–736.
[46] R. G. Thurman and P. Scholz (1973). Interaction of mixed-function oxidation with biosynthetic processes. *European Journal of Biochemistry*, **38**, 73–78.
[47] C. Waydhas, K. Weigl and H. Sies (1978). The disposition of formaldehyde and formate arising from drug N-demethylation dependent on cytochrome P-450 in hepatocytes and in perfused rat liver. *European Journal of Biochemistry*, **89**, 143–150.
[48] R. R. Erickson and J. L. Holtzman (1976). Kinetic studies on the metabolism of ethylmorphine by isolated hepatocytes from adult rats. *Biochemical Pharmacology*, **25**, 1501–1506.
[49] T. S. LeGrand, and T. Y. Aw (1997). Chronic hypoxia, glutathione-dependent detoxication and metabolic instability in rat small intestine. *American Journal of Physiology*, **272**, G328–G334.
[50] C. A. Rhoads and T. Y. Aw (1997). Persistent lipid peroxide challenge *in vivo* compromises GSH redox cycle function and peroxide detoxication in enterocytes. *FASEB Journal*, **11**(3), A220.
[51] T. S. LeGrand and T. Y. Aw (1998). Chronic hypoxia alters utilization of glucose during GSH-dependent detoxication in rat small intestine. *American Journal of Physiology* **274**, G376–G384.
[52] B. Burrows, L. J. Kettel, A. H. Niden, M. Rabinowitz and C. R. Diener (1972). Patterns of cardiovascular dysfunction in chronic obstructive pulmonary disease. *New England Journal of Medicine*, **286**, 912–918.

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