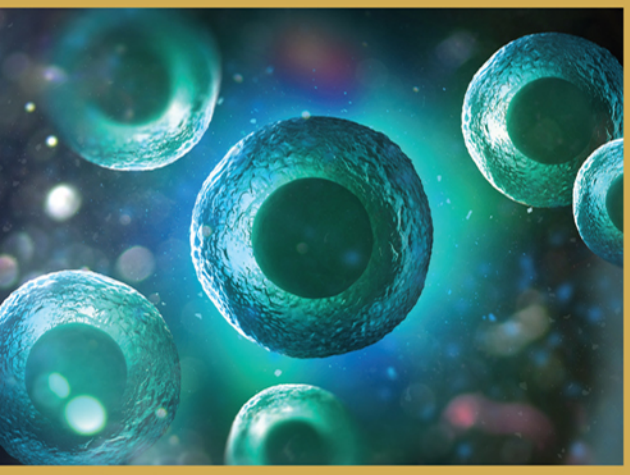

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Oxidative Stress and Dietary Antioxidants

Second Edition

Edited by
VICTOR R. PREEDY

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SECOND EDITION

Edited by

VICTOR R. PREEDY

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Preface

In the past few decades there have been major advances in our understanding of the etiology of disease and its causative mechanisms. Increasingly it is becoming evident that free radicals are contributory agents: either to initiate or propagate pathologies or to create an overall cellular and metabolic imbalance. Furthermore, a reduced intake of dietary antioxidants can also lead to an increased risk of specific diseases. On the other hand, there is abundant evidence that naturally occurring antioxidants can be used to prevent, ameliorate, or impede such disease risks. The science of oxidative stress and free radical biology is rapidly advancing and new approaches include examining the roles of genetics and molecular biology.

However, most textbooks on dietary antioxidants do not have material on the fundamental biology of free radicals, especially their molecular and cellular effects on pathology. They also fail to include material on the nutrients and foods that contain antioxidative activity. In contrast, most books on free radicals and disease have little or no text on the usage of natural antioxidants.

In the present volume *Diabetes: Oxidative Stress and Dietary Antioxidants, Second Edition*, holistic information is imparted within a structured format of three main sections.

Section I: Oxidative Stress and Diabetes

Section II: Antioxidants and Diabetes

Section III: Techniques and Resources

Section I: Oxidative Stress and Diabetes covers the basic biology of oxidative stress from molecular biology to physiological pathology. In *Section II: Antioxidants and Diabetes* we describe agents and their actions. The caveat of these chapters in *Section II* is that there needs to be further in-depth analysis of these components in terms of safety and efficacy as some material is exploratory or preclinical. A cautionary and critical approach is needed. Nevertheless, the material in *Section II* can provide the framework for further in-depth analysis or studies. This would be via well-designed clinical trials or via the analysis of pathways, mechanisms, and components in order to devise new therapeutic strategies. *Section III: Techniques and Resources* provides a practical source of information. Both preclinical and clinical studies are embraced using an evidence-based approach. However, the science of oxidative stress is not described in isolation but in concert with other processes such as apoptosis, cell signaling, and receptor-mediated responses. This approach recognizes that diseases are often multifactorial and oxidative stress is a single component of this.

Diabetes: Oxidative Stress and Dietary Antioxidants, Second Edition is designed for dietitians and nutritionists, food scientists, as well as healthcare workers and research scientists. In this book the target audience also includes diabetologists, biochemists and food scientists, clinicians, basic science researchers, medical students, healthcare industry workers, endocrinologists, family medicine physicians, diabetes nurse practitioners, and drug developers. Contributions are from leading national and international experts including those from world-renowned institutions.

Professor Victor R. Preedy,
King's College London

CHAPTER

3

Diabetic enteric neuropathy: imbalance between oxidative and antioxidative mechanisms

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List of abbreviations

ENS	enteric nervous system
HO	heme oxygenase
IR	immunoreactive
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
ROS	reactive oxygen species
STZ	streptozotocin

Structure, function, and diabetic state of the enteric nervous system

The gastrointestinal tract differs from all other organs in that it has an intrinsic nervous system known as the enteric nervous system (ENS).[1] The ENS has compound functions: controlling the movement of the gastrointestinal tract and gastric acid secretion, regulating movement of fluid across the epithelium and local blood flow, modifying nutrient absorption, interacting with the endocrine and immune systems of the gastrointestinal tract, and maintaining the integrity of the epithelial barrier between the intestinal lumen and tissues within the gut wall.[2]

Enteric neurons, along with the enteric glia cells, are arranged in networks of enteric ganglia connected by interganglionic strands.[3] The enteric ganglia are organized into two main plexuses in the intestinal wall. The myenteric plexus is between the outer longitudinal and circular muscle layers and extends the full length of the digestive tract from the esophagus to the rectum. The main function of the myenteric plexus is the regulation of the gastrointestinal motility. The submucous plexus is prominent only in the small and large intestines. Submucous ganglia reside in the submucosa tissue layer—in small animals in one layer, in larger animals in two layers. This plexus regulates absorption, blood flow, secretion in the gut wall, and fluid movement between the lumen and the intestinal epithelia.[4,5]

The total number of enteric neurons in humans is 200−600 million, which is approximately equal to the number of neurons in the spinal cord.[5] Enteric neurons are highly varied in their morphological, neurochemical, and functional properties (Fig. 3.1). Intrinsic primary afferent neurons, interneurons, and motor neurons are all present in the ENS and form local neural circuits in the gastrointestinal tract.[4,5] The ENS can work autonomously: it communicates bidirectionally with the central nervous system and the other two divisions of the peripheral nervous system—the sympathetic and parasympathetic divisions. This bidirectional connection between the ENS and central nervous system is known as the gut−brain axis.[6]

The enteric glia cells closely associated with the neurons resemble the astrocytes of the central nervous system rather than Schwann cells. In enteric neurons, similarly to the neurons of the central nervous system, several neurotransmitters and neuromodulators are present. Nonadrenergic-noncholinergic neurotransmission, via vasoactive intestinal polypeptide, nitric oxide (NO), and substance P, plays a significant role in the

Diabetes.
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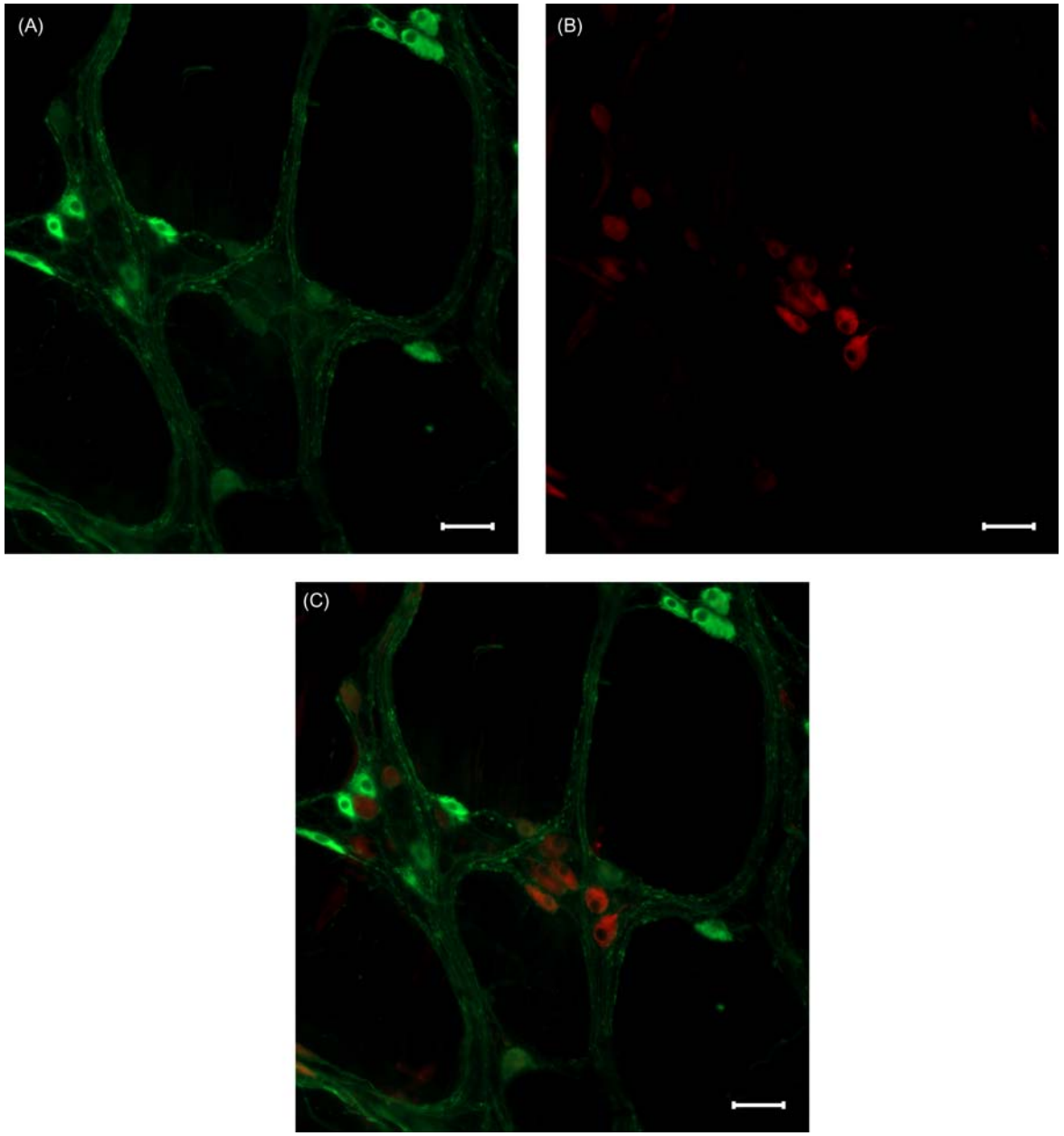

FIGURE 3.1 Photomicrographs of neuronal nitric oxide synthase (*A*) neurofilament 200 (*B*) immunostained myenteric neurons in a whole-mount preparation from the colon of a control rat. Figure C shows the merged pictures. Scale bars: 50 μm.

peristaltic reflex of the gastrointestinal tract.[4,7] In nitrergic enteric neurons, NO is produced by the neuronal NO synthase (nNOS) enzyme. The ratio of nitrergic neurons to the total number of neurons is moderate in the submucous plexus, while it is higher in the myenteric ganglia and varies between 25% and 50% in the different gut regions and species[4,8] (Fig. 3.2).

Numerous reports in the literature have suggested that nitrergic myenteric neurons are especially susceptible to neuropathy in different pathological states like alcoholism,[9] mitochondrial dysfunction,[10] ischemia,[11] or diabetes.[12–15]

The review of Cellek et al. discusses two phases of nitrergic enteric neuropathy.[15] The first phase, with the loss of nNOS in the neurons and nitrergic dysfunction, is reversible on insulin replacement. The second phase is characterized by neuronal apoptosis and is irreversible on insulin replacement. In the past decade it has become clear that the development of the diabetic nitrergic neuropathy is more complicated than suggested earlier[15] and differs from segment to segment along the gastrointestinal tract.[13]

The imbalance between prooxidant mechanisms and antioxidant defenses contributes to the oxidative

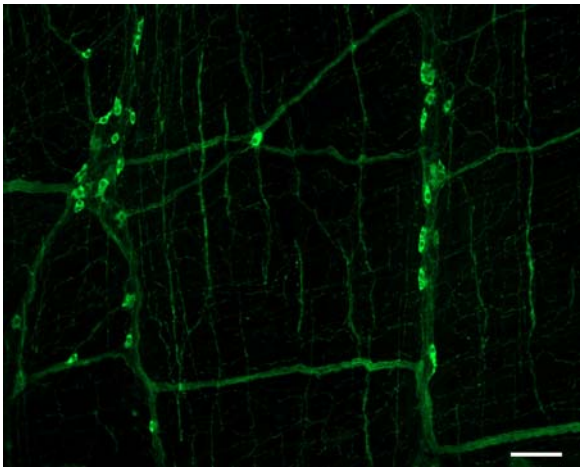

FIGURE 3.2 Photomicrograph of neuronal nitric oxide synthase–immunostained myenteric neurons in a whole-mount preparation from the duodenum of a control rat. The number of nitrergic neurons in notable in the myenteric plexus. The main function of the nitrergic myenteric neurons is the regulation of the gastrointestinal motility. Scale bar: 100 μm.

stress in a diabetic state. Elevated oxidative stress is the result of hyperglycemia-induced increased reactive oxygen species (ROS) generation and the impairment of endogenous defenses promoting the pathogenesis of diabetes. Oxidative stress appears to be crucial in diabetes-related enteric neuropathy and gastrointestinal complications. Oxidative stress not only activates different cellular pathways, but also initiates and amplifies neuroinflammation due to the production of proinflammatory cytokines.[16] Antioxidants have different mechanisms to ameliorate nerve dysfunction in diabetes by acting directly against oxidative damage.[17]

Gut region-specific oxidative environment and antioxidant capacity under physiological conditions

It is well-known that the different parts of the gastrointestinal tract are anatomically and functionally different. This regionality of the intestinal structure and function develops under strict genetic control[18,19] and may contribute to the unique features of the enteric neurons under physiological or pathological conditions in different gut segments.

During food consumption, in addition to a range of antioxidants, oxidative agents also enter the body, so the intestine fulfills a critical role in the regulation and maintenance of the antioxidant-prooxidant balance.[20,21] The appropriate antioxidant defense allows cells to survive in an oxygenated environment. Therefore the redox status of the different gut segments is extremely important in health and in many metabolic diseases.[22]

In the duodenum, an adequate antioxidative environment ensures the normal metabolism of cells. In this particular gut segment in the chicken, high concentrations of vitamin E were present in the mucosa which decreased toward the ileum and colon.[23] Similarly, the highest concentrations of carotenoids were observed in duodenal mucosa, with much lower levels in the ileum and colon.[23] The total antioxidant activity, as well as the superoxide dismutase and catalase activity, was also higher in the rat small intestinal mucosa than in the colon.[24] Glutathione, which is considered to be an active antioxidant, was found in high concentration in the duodenum.[25] In addition, the high level of heme oxygenase 1 (HO1) and HO2 expression in tissue homogenates of the duodenum (originated from the smooth muscle layers and the myenteric plexus) and the high percentage (88%) of HO1-expressing myenteric ganglia in the duodenum, also pointed to a protective basal microenvironment.[14] Microsomal HO activity was also the highest in the duodenal mucosa, where the absorption of hemoglobin iron is more effective than in the caudal intestinal segments.[26] Furthermore a number of *Lactobacillus* species as probiotic strains were observed in high relative abundance in duodenal microbiota originated from luminal content.[27–29] These findings suggest that as a result of explicit antioxidant capacity of duodenum, the cells located there have greater tolerance and protection against oxidative stress.

Under physiological conditions the expression of the HO proteins is extremely low in the myenteric ganglia of the ileum; only half of the ileal ganglia contained HO1–immunoreactive (IR) neurons and from these ganglia only 16% contained nNOS–HO1 colocalized neurons. Furthermore, the number of HO–IR or nNOS–HO–IR cells was also lowest in the ileum compared to other gut segments.[14] In correlation with this, others revealed that only 10% of neurons in the rat ileum[30] are nNOS–HO2–IR and that HO1 protein expression is hardly detectable in the ileal mucosa. Moreover, it is proved that HO1–IR and HO2–IR neurons are present in very small amounts in the submucous plexus of the small intestine.[31] The slight expression of these antioxidants may contribute to significantly lower protection against different pathological stimuli in the ileum.

The region-specific excess of bacteria in the gut determines the oxygen supply of the small and large intestine[22,32,33] resulting a deep anaerobic state in the distal segments.[28] For example, in the distal ileum and the colon, the presence of “nonpathogenic” anaerobic bacteria *Veillonella* sp. has great dominance.[27] It is also supposed that in the colon, where the baseline redox

status is far from optimal, the physiological expression of HO1 and HO2 is the most pronounced in the colonic myenteric ganglia.[14,34] As a preconditioning factor, the HO enzymes are also abundant in the submucous neurons of the colon.[31,34] Other results also showed[32] that the colon generates more ROS than does the small intestine, and this prooxidant environment may contribute to greater cancer susceptibility.[32]

Diabetes-related changes in the expression of oxidants and antioxidants in the enteric ganglia of different gut segments

We have demonstrated that nitrergic myenteric neurons located in different gut segments display different susceptibilities to diabetic damage (Fig. 3.3) and insulin treatment.[13,35] These findings emphasize the importance of the neuronal microenvironment along the gastrointestinal tract in the pathogenesis of diabetic nitrergic neuropathy and urge investigation of the underlying molecular mechanisms, like region-specific intestinal ROS accumulation and endogenous antioxidant distribution.

Recent studies[14,36] have demonstrated evidence for gut region-specific accumulation of ROS, and have also shown that enhanced oxidative stress leads to regionally distinct activation of endogenous antioxidants in the different intestinal segments of rats with streptozotocin (STZ)-induced diabetes (Fig. 3.4).

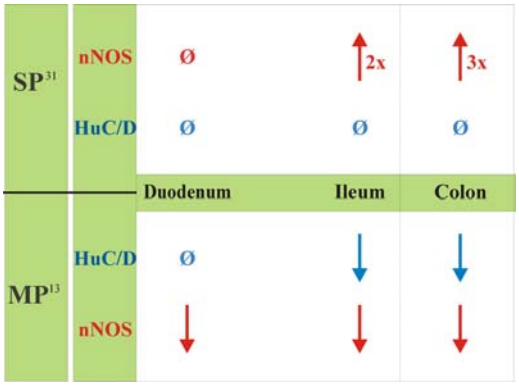

FIGURE 3.3 Density of total and nitrergic neurons in the two enteric plexuses and different intestinal regions of diabetic rats. The number of total and nitrergic neurons varied differently in the submucous and myenteric plexuses (SP and MP) of diabetics. The total number of submucous neurons was not affected in the different gut segments, while with the exception of the duodenal ganglia, the number of nitrergic neurons was increased significantly in the ileum and colon by diabetes. In the myenteric ganglia, a gut region-specific decrease in total and nitrergic neuronal density was demonstrated. Summarized from Bódi et al. (2017)[31] and Izbéki et al. (2008).[13] HuC/D is a pan-neuronal marker of enteric neurons; nNOS-neuronal nitric oxide synthase.

Duodenum

In our study, in the duodenum of type 1 diabetic rats, the number of nitrergic myenteric neurons decreased, while the total neuronal number was not altered, suggesting that only the neurochemical character of the cells changed and no apoptosis occurred.[13] Coincidentally, there were no significant changes in the production of a powerful oxidant, peroxynitrite, whereas the mRNA level of the free radical scavenger metallothionein-2 increased ~300-fold in this particular gut segment. Additionally, 2.5–3-fold elevated glutathione levels were revealed in the duodenal tissues of diabetics, which may protect cellular proteins against oxidation, directly detoxify ROS, and play a remarkable role to maintain the optimal thiol/redox balance.[36] Moreover, the highest level of HO1 and HO2 expression in tissue homogenates of control duodenum also emphasizes a highly protective microenvironment in this intestinal segment.[14] It is assumed that due to the adequate oxidative environment, the nitrergic neurons receive greater protection and can better tolerate hyperglycemia-related oxidative stress in the duodenum. In this gut segment, besides a decrease in the number of nNOS neurons, the number of nNOS–HO colocalized myenteric neurons was not altered significantly. This suggests that

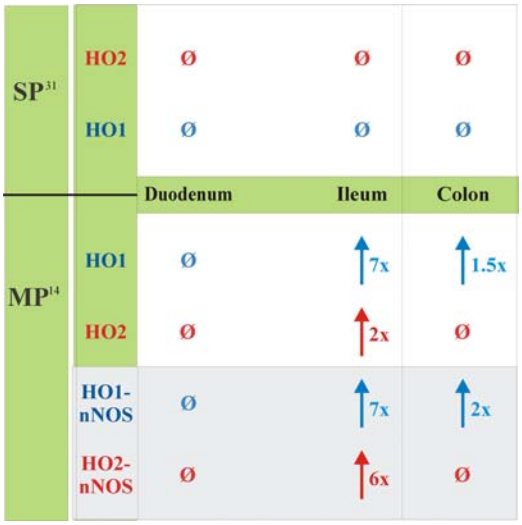

FIGURE 3.4 Expression of endogenous heme oxygenase 1 and 2 in the two enteric plexuses and different intestinal regions of diabetic rats. In diabetics, the number of heme oxygenase (HO) 1 and HO2-immunoreactive neurons did not change significantly in the submucous plexus (SP) of different intestinal segments compare to controls. However, in the myenteric plexus (MP) of diabetic rats, the number of HO1- and HO2-positive neurons, as well as the number of those neurons in which the HO is colocalized with neuronal nitric oxide synthase (HO1-nNOS and HO2-nNOS) increased significantly in the ileum and colon, but not in the duodenum. Summarized from Bódi et al. (2017)[31] and Chandrakumar et al. (2017).[14]

HO-containing nitrergic neurons are less affected by diabetic damage.[14]

Previous studies have shown that diabetic impairments of the intestinal microbiota contribute to the imbalance between the accumulation of reactive radicals and endogenous antioxidant defenses.[37–39] In our study with STZ-induced diabetic rats, using next-generation DNA sequencing, the duodenal microbiota did not display the development of a disadvantageous environment. Moreover, in the microbial community of the diabetic duodenum, 49% of the total reads were due to the order *Lactobacillales* (including almost all members of the genus *Lactobacillus*), relative to 31% in healthy controls.[28] The increased number of lactic acid bacteria strains, the key players of probiotics, results in enhanced antioxidant capacity in different ways (e.g., these probiotics produce antioxidant metabolites, regulate different signaling pathways, downregulate activities of ROS-producing enzymes, or improve the absorption of antioxidants and reduce postprandial lipid concentrations).[29,40] It has also been observed that consumption of these probiotics presented higher activity of superoxide dismutase and glutathione peroxidase in diabetic patients relative to controls.[41]

The appropriate intracellular glutathione level is important to maintain a proper intestinal Ca^{2+} absorption.[42,43] It appears that the duodenum is the main site of that because the lowest pH of the gut with decreasing absorption rate to distal part.[44] In mice on a high-fat diet, increased oxidative stress and redox imbalance was revealed in the duodenum, resulting in the inhibition of calcium absorption and related gene expression.[45] Similarly, in STZ-induced diabetic rats, it was also demonstrated that intestinal oxidative stress at early stages of diabetes leads to an inhibited Ca^{2+} absorption. However, time-dependent adaptive mechanisms contribute to normalizing the intestinal Ca^{2+} absorption, as well as the duodenal redox state.[43,46]

Ileum

In the diabetic ileum, not only did the density of nitrergic myenteric neurons decrease, but so did the total number of neurons.[13,35,47] In this particular gut segment, the markers of oxidative stress caused by constant hyperglycemia were markedly expressed. The level of malondialdehyde, an end product of lipid peroxidation, was almost doubled, while the levels of antioxidant molecules, such as superoxide dismutase, catalase, and glutathione, were significantly lower in ileal tissue homogenates of diabetic rats compared to controls.[48] Similarly, significantly increased lipid peroxidation and protein oxidation was observed in another study using diabetic rats.[49]

Shotton and Lincoln[50] have demonstrated an increased cell body size of nNOS−IR neurons in diabetes, while HO2−IR neurons were not affected. Moreover, the double-labeling studies revealed that the diabetes-related alteration in size of perikarya was confined to those nNOS−IR neurons that did not contain HO2; those nitrergic neurons were protected against diabetic effects, in which nNOS and HO2 were colocalized. Interestingly, compared to the extremely low presence of the HO proteins in controls, all of the ileal ganglia included HO1−IR neurons and more than 60% of them were also IR for nNOS in diabetic rats. The greatest increase in the ratio of nNOS−HO2−IR ganglia was also shown in the ileum of diabetics[14] compared to other intestinal regions. Furthermore, both the HO1- and the nNOS−HO1−IR neuronal number was enhanced sevenfold, and the number of nNOS−HO2−IR neurons increased sixfold in the diabetic ileum[14] compared to controls. This data supports that many of the nitrergic neurons start to produce HO enzymes and suggests that those nNOS-positive neurons which are not colocalized with HOs will be injured by diabetes.

Based on these findings, the highest increase in expression of the endogenous HO system and the colocalization of HO1 and HO2 with nNOS in myenteric neurons was observed in the ileum of diabetics, which highlights the outstanding concern of this intestinal segment in diabetes-related damage. This remarkable diabetic involvement of the ileum was also predicted in our earlier study.[28] We demonstrated that only the diabetic ileal feces samples exhibited a massive (more than 30%) *Klebsiella* invasion.[28] Accumulation of these pathogens results in gut inflammation, leaky epithelium and easy paths for bacteria through the intestinal tissues, developing a pathological microenvironment and impairment of gut immunity.[51] It is assumed that diabetes-related explicit changes in the microbial composition of the ileum[28] may contribute to the elevated mucosal immune response and the greatest induction of endogenous HO defenses in this segment. It was also reported that intestinal HO1 is induced by the enteric microbiota and regulates macrophage activity,[52] which emphasizes even further the importance of a disturbed enteric microbiota in the determination of intestinal redox status. Ileal microbiota dysbiosis is responsible for the glucagon-like peptide-1 resistance, and therefore obstructs glucagon-like peptide-1-induced NO production by enteric neurons and induces enteric neuropathy in diabetic mice.[53]

Colon

In the colon of diabetic rats, both the nitrergic and the total neuronal number decreased significantly.[13,35]

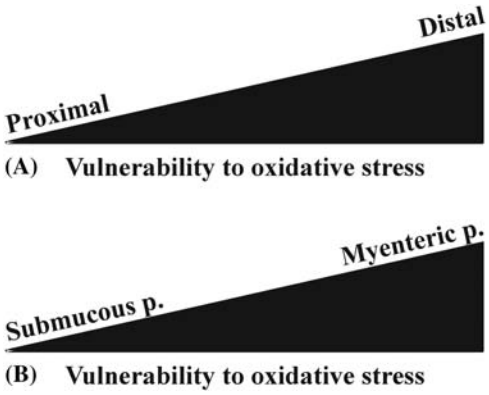

FIGURE 3.5 Vulnerability of the gut and enteric plexuses to diabetes-related oxidative stress. The distal part of the gut is more vulnerable than the proximal to diabetic oxidative stress (A). While myenteric neurons are more susceptible to diabetic damage, the submucous ganglia have greater resistance against hyperglycemia-induced oxidative stress (B). p-plexus.

In correlation with this myenteric cell loss, peroxynitrite production was doubled. Superoxide dismutase activity decreased, whereas glutathione level, catalase activity, and metallothionein-2 expression were not significantly changed in the large intestine of diabetic animals.[36] The level of metallothionein-1 mRNA was increased sevenfold in the colon. In the colon of diabetics, the presence of severe necrosis was also confirmed by electron microscopy.[36] These data further suggest that the distal part of the gut is the most vulnerable segment to oxidative stress (Fig. 3.5A).

The proportion of nNOS−IR neurons colocalizing with HO1 exhibited a threefold increase (72% vs. 23%) and the ratio of nNOS−IR neurons colocalizing with HO2 was also enhanced (68% vs. 44%) in the colon of diabetics.[14] Besides a 22% decrease in the nNOS neuronal number, a more than 50% increase was demonstrated in the number of nNOS−HO1−IR neurons, while the number of nNOS−HO2−IR neurons did not alter significantly compared to controls. This suggests that HO-containing nitrergic neurons enjoy higher protection, while those that do not contain HO are heavily affected by diabetic damage.

Bacterial dysbiosis was also demonstrated in the large intestinal segment of diabetics in our study.[28] Regarding the microbial composition of the diabetic colon, the major representatives belonged to the genus *Klebsiella*. The relative abundance of 6% of this genus was significantly less than that reported in the diabetic ileum, but still noteworthy compared to the controls.[28] This observed increase in the level of the pathogen *Klebsiella* could be associated with intestinal inflammation and enteric neuropathy.

Submucous ganglia

The involvement of different myenteric neuronal populations in diabetic oxidative injuries has been thoroughly investigated in human and animal models. However, the responsiveness of submucous neurons to diabetic damage and the state of their antioxidant defenses is poorly studied. It has been recently demonstrated that the total number of submucous neurons was not affected by diabetes in different segments of the small and large intestine.[31,54] These findings suggest greater resistance of submucous neurons against hyperglycemia-induced oxidative stress[31,55] (Fig. 3.5B). The density of the nitrergic submucous population did not vary in the different intestinal segments.[31,56] In the duodenal submucous ganglia, the number of nitrergic neurons was not affected by diabetic state. However, in the ileum and colon, it increased significantly, presumingly due to modifications of neurochemical coding as an answer to diabetic oxidative damage (Fig. 3.3). Increased immunoreactivity in vasoactive intestinal polypeptide-positive neurons in the submucous plexus has also been revealed in diabetes.[57] Treatment with different antioxidant agents, like ascorbic acid or quercetin proved to be neuroprotective against these diabetes-related alterations.[55,57] The distribution of HO1−IR and HO2−IR submucous neurons were more pronounced in the large intestine (about 50%) than in the small intestinal segments (0%−5%) in healthy controls. Chronic hyperglycemia did not result in any significant changes in HO-immunoreactivity in these segments, while these neurons had intestinal region-dependent responsiveness to immediate insulin treatment.[31] As colocalization of nNOS−HO2 in submucous neurons was observed in other studies,[34,58] drawing attention to its protective capacity, the endogenous HOs may contribute to the elevated number of nitrergic submucous neurons in the distal part of the gut, which requires further studies.

Conclusion and perspectives

Considering the above-mentioned results, the imbalance between oxidative and antioxidative mechanisms in diabetes intensely contributes to enteric neuropathy in the gastrointestinal tract. It is also important to emphasize that the two enteric plexuses are affected by the hyperglycemia-related oxidative stress differently and in a strictly gut region-specific manner (Fig. 3.6).

Further highlighting the importance of oxidative and antioxidative imbalance, other studies show that oxidative stress plays a pivotal role in pathological states where the gastrointestinal tract is injured, like

Oxidative stress		Antioxidant defence
No changes in peroxynitrite production[36]	**Duodenum**	Metallothionein mRNA ↑[36] Glutathione ↑[36] HO-containing nitrergic neurons are less damaged[14] Higher representation of order *Lactobacillales*[28]
Lipid peroxidation ↑[48,49] Protein oxidation ↑[49] *Klebsiella* invasion[28] Microbiota dysbiosis[28,53]	**Ileum**	Superoxide dismutase, catalase, glutathione ↓[48] Greatest induction of HO1 and HO2 expression in nitrergic neurons[14]
Peroxynitrite ↑[36] Microbiota dysbiosis[28]	**Colon**	Superoxide dismutase ↓[36] Catalase, glutathione Ø[36] Metallothionein 1 mRNA ↑[36] Induction of HO system in nitrergic neurons[14]

FIGURE 3.6 Diabetes-related changes of oxidative stress markers and antioxidant defense mechanisms in the different gut segments. In the diabetic duodenum, besides the elevated levels of antioxidant enzymes, the higher abundance of the order *Lactobacillales* was observed. In contrast, enhanced lipid peroxidation, protein oxidation, a massive invasion of pathogens, *Klebsiella* and microbial dysbiosis were demonstrated in the distal part of the gut. In addition, the greatest induction of the endogenous heme oxygenase (HO) system was revealed in the ileum and colon.

gut inflammation, aging,[7] gastrointestinal mucosal disease,[59] or alcoholism.[60]

Recent data have elucidated that the gut microbiota is a key contributor to the pathophysiological effects of the gut–brain axis.[6] Therefore the imbalance between oxidative elements and antioxidant defenses not only has an important local effect in the gastrointestinal tract, but also has a unique function in the development of neurodegenerative or neuropsychological disorders.

Summary points

- Nitrergic myenteric neurons in different gut segments display different susceptibilities to diabetic damage and to insulin treatment, emphasizing the importance of the neuronal microenvironment in the pathogenesis of diabetic neuropathy.
- Shifts in the balance between the production and scavenging of free radicals lead to region-specific oxidative stress in the gut, which in turn contribute to enteric neuropathy in diabetes.
- Both the accumulation of reactive oxygen species and the activation of endogenous antioxidants show distinct regional differences in diabetes.
- The distal part of the small intestine shows greater changes to oxidative stress than the proximal part.
- Nitrergic neurons that contain heme oxygenase enjoy higher protection while those that do not contain heme oxygenase are heavily affected by oxidative damage.
- Microbial dysbiosis demonstrated in the distal part of the gut may contribute to inducing endogenous heme oxygenase defense mechanisms in the ileum and colon.

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