

DIABETES

Oxidative Stress and Dietary Antioxidants

Second Edition

Edited by
VICTOR R. PREEDY



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SECOND EDITION

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VICTOR R. PREEDY

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London, United Kingdom*



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Contents

List of Contributors	xiii	A new antioxidant delivery	21
Preface	xvii	Conclusion	21
		Summary points	22
		References	22
 Section I			
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Oxidative stress and diabetes			
1. Oxidative stress markers in diabetes	3	3. Diabetic enteric neuropathy: imbalance between oxidative and antioxidative mechanisms	25
EUGENE BUTKOWSKI		NIKOLETT BÓDI AND MÁRIA BAGYÁNSZKI	
List of abbreviations	3	List of abbreviations	25
Introduction	3	Structure, function, and diabetic state of the enteric nervous system	25
Oxidative stress: an overview	4	Gut region-specific oxidative environment and antioxidant capacity under physiological conditions	27
Oxidative stress in type 2 diabetes mellitus and cardiovascular disease	4	Diabetes-related changes in the expression of oxidants and antioxidants in the enteric ganglia of different gut segments	28
Protein kinase C and reactive oxygen species	6	Conclusion and perspectives	30
Reduced glutathione, glutathione disulfide, and glutathione/glutathione disulfide	6	Summary points	31
8-Hydroxy-2'-deoxyguanosine	6	References	31
F ₂ -isoprostanes	7		
Malondialdehyde	7	4. Hyperglycemia-induced oxidative stress in the development of diabetic foot ulcers	35
Whole blood viscosity	8	ELIZABETH BOSEDE BOLAJOKO, OLUBAYO MICHAEL AKINOSUN AND AYE AYE KHINE	
Inflammatory biomarkers	8	List of abbreviations	35
The interleukins	9	Introduction	35
Insulin-like growth factor-1	9	Generation of oxidative stress	36
Hyperglycemia and coagulability	9	Hyperglycemia-induced oxidative stress in the development of foot ulcer and delayed wound healing in people with diabetes mellitus	41
Conclusion	9	Summary points	46
Summary points	9	References	47
References	10		
2. Oxidative stress and diabetic neuropathy	13	5. Oxidative stress in diabetic retinopathy	49
HAJUNG CHUN AND YONGSOO PARK		JOSE JAVIER GARCIA-MEDINA, VICENTE ZANON-MORENO, MARIA DOLORES PINAZO-DURAN, ELISA FOULQUIE-MORENO, ELENA RUBIO-VELAZQUEZ, RICARDO P. CASAROLI-MARANO AND MONICA DEL-RIO-VELLOSILLO	
List of abbreviations	13	List of abbreviations	49
Introduction	13	Histopathology of diabetic retinopathy	49
Natural history	14	Oxidative stress mechanisms	52
Hyperglycemia is a crucial cause of diabetic neuropathy	15	Oxidative stress and diabetic retinopathy	53
Oxidative stress is a key mediator of diabetic neuropathy	15	Summary points	55
Role of endoplasmic reticulum, microRNAs, and mitochondria in the pathogenesis of diabetic peripheral neuropathy	17	References	56
Normal antioxidant defense mechanisms	18		
Role of interventions in endogenous antioxidant signaling	19		
Role of exercise and diet	20		
Clinical trials of antioxidants	20		

6. Cerebral ischemia in diabetics and oxidative stress	59	10. MicroRNAs linking oxidative stress and diabetes	97
SUNJOO CHO, PERRY FUCHS, DEEPANEETA SARMAH, HARPREET KAUR, PALLAB BHATTACHARYA AND KUNJAN R. DAVE		JULIAN FRIEDRICH AND GUIDO KRENNING	
List of abbreviations	59	List of abbreviations	97
Introduction	59	Introduction	97
Conclusion	66	MicroRNA biogenesis and function	98
Summary points	66	The influence of oxidative stress on microRNA biogenesis	98
References	67	The influence of microRNAs on oxidative stress in diabetes	99
		RedoximiRs in diabetes	101
		MicroRNAs and oxidative stress in specific diabetic complications	102
7. Gingival wound healing in diabetes	69	miR-126 and the diabetic vasculature	102
PRIMA BURANASIN, KENGO IWASAKI AND KOJI MIZUTANI		miR-25 and diabetic nephropathy	104
List of abbreviations	69	miR-15a and diabetic retinopathy	104
Introduction	69	Conclusion and perspective	104
In vitro study	70	Summary points	104
In vivo study	71	References	105
Clinical findings	74		
Conclusion	75	11. Polymorphism of MnSOD 47C/T antioxidant enzymes and type 1 diabetes	107
Summary points	75	A. EDDAIKRA AND C. TOUIL BOUKOFFA	
References	76	List of abbreviations	107
		Introduction	107
8. Oxidative stress in gestational diabetes mellitus	79	Antioxidant defenses	108
PHUDIT JATAVAN		Gene of superoxide dismutase 2	108
List of abbreviations	79	MnSOD 47C/T polymorphism	109
Introduction	79	Minor allele of MnSOD 47C/T	110
Oxidative stress in gestational diabetes mellitus	80	Role of the manganese	111
Diabetic embryopathy	80	Role of hydrogen peroxide	111
Oxidative stress in the amniotic fluid and placenta in gestational diabetes mellitus patients	81	Mitochondrial production of reactive oxygen species	111
Oxidative stress level in fetal circulation in pregnancy affected with gestational diabetes mellitus	82	Regulation of the superoxide dismutase 2 gene	111
Role of antioxidant supplements in gestational diabetes mellitus	82	Concept of adaptative response	112
Fetal and maternal outcomes	83	Oxidation of proteins	112
Summary points	83	Conclusion	113
References	83	Summary points	113
		References	113
9. Epigenetics, oxidative states and diabetes	87	Further reading	115
ELEONORA SCACCIA, ANTONELLA BORDIN, CARMELA RITA BALISTRERI AND ELENA DE FALCO			
List of abbreviations	87	12. Sodium-glucose cotransporter 2 inhibitors, diabetes, and oxidative stress	117
Introduction	87	SEBASTIAN STEVEN, KATIE FRENIS, MATTHIAS OELZE, KSENIJA VUJACIC-MIRSKI, MARIA TERESA BAYO JIMENEZ, SANELA KALINOVIC, SWENJA KRÖLLER-SCHÖN, THOMAS MÜNDEL AND ANDREAS DAIBER	
Epigenetics controls physiological mechanisms modulated by redox states	88	List of abbreviations	117
The role of mitochondria: the hotbed of redox states	90	Global burden of disease and mortality	117
MicroRNAs: the epigenetic regulators between redox states and diabetes	91	Prevalence and incidence of diabetes, treatment options as well as its contribution to cardiovascular disease and mortality	118
Diabetes changes the redox states through epigenetics	93	Pathomechanisms of diabetes	119
Summary points	94	Experimental studies in type 1 and type 2 diabetic rats with sodium-glucose cotransporter 2 inhibitor therapy	120
References	94	Summary points	125
		Acknowledgments	126
		Conflicts of interest	126
		References	126

13. NADPH oxidases, nuclear factor kappa B, NF-E2-related factor2, and oxidative stress in diabetes	129	Introduction: oxidative stress in diabetes	161
ANDRZEJ BERESEWICZ		Docosahexaenoic acid	162
List of abbreviations	129	Docosahexaenoic acid and oxidative stress	163
Introduction	129	Docosahexaenoic acid derivatives: a new frontier	164
Cellular signaling via redox modification of target proteins	130	Docosahexaenoic acid and oxidative stress in the brain	165
Vascular sources of reactive oxygen and nitrogen species	130	Summary points	166
Nuclear factor kappa B and NF-E2-related factor2 are controlled by reactive oxygen species and control reactive oxygen species	132	References	166
The organization of redox-signaling networks in the vasculature	133		
NADPH oxidases, nuclear factor kappa B, NF-E2-related factor2, and endothelial form of nitric oxide synthase in diabetes	134	17. Antioxidant supplementation in diabetic retinopathy	169
Summary points	135	JOSE JAVIER GARCIA-MEDINA, ELENA RUBIO-VELAZQUEZ, RICARDO P. CASAROLI-MARANO, VICENTE ZANON-MORENO, MARIA DOLORES PINAZO-DURAN, ELISA FOULQUIE-MORENO AND MONICA DEL-RIO-VELLOSILLO	
References	135	List of abbreviations	169
		Introduction	169
14. Antioxidant properties of drugs used in type 2 diabetes management	139	In vitro studies	170
SIU-WAI CHOI AND CYRUS KIN-CHUN HO		Animal studies	178
List of abbreviations	139	Clinical studies	179
Introduction	139	Final comments and future directions	181
Conclusion	147	Summary points	181
Summary points	147	References	182
References	147		
		18. <i>Basella alba</i> , oxidative stress, and diabetes	187
		DENNIS S. AROKOYO AND OLUBAYODE BAMIDELE	
		List of abbreviations	187
		Introduction	187
		Beneficial effects of <i>Basella alba</i> in diabetes mellitus	191
		Conclusion	192
		Summary points	192
		References	192
		19. <i>Bauhinia vahlii</i> and antioxidant potential in diabetes	195
		ENGY A. MAHROUS AND MOHAMMED M. NOOH	
		List of abbreviations	195
		Introduction	195
		Conclusion	200
		Summary points	200
		References	201
		20. Carnosine, pancreatic protection, and oxidative stress in type 1 diabetes	203
		VITALE MICELI AND PIER GIULIO CONALDI	
		List of abbreviations	203
		Introduction	203
		Conclusion	209
		Summary points	209
		References	210

Section II

Antioxidants and diabetes

15. Antioxidants, oxidative stress, and preeclampsia in diabetes	151		
ARPITA BASU AND TIMOTHY J. LYONS			
List of abbreviations	151		
Introduction	151		
Oxidative stress and antioxidant status in pregnancies complicated by type 1 diabetes mellitus and gestational diabetes mellitus	152		
Oxidative stress and antioxidant status in preeclampsia	153		
Antioxidant supplementation in preeclampsia and gestational diabetes mellitus: findings from clinical studies	155		
Summary points	157		
References	157		
16. Antioxidative component of docosahexaenoic acid in the brain in diabetes	161		
DANIEL LÓPEZ-MALO, EMMA ARNAL, MARIA MIRANDA, SIV JOHNSEN-SORIANO AND FRANCISCO J. ROMERO			
List of abbreviations	161		

21. <i>Centella asiatica</i> : its potential for the treatment of diabetes	213	Cranberry and gut microbiota homeostasis	247
AYODEJI B. OYENIHI, BLESSING O. AHIANTE, OMOLOLA R. OYENIHI AND BUBUYA MASOLA		Cranberry, enhanced intestinal barrier integrity, and decreased metabolic endotoxemia	248
List of abbreviations	213	Cranberry, glucose metabolism, and insulin sensitivity	249
Introduction	213	Conclusion	250
Oxidative stress, diabetes, and diabetic complications	214	Summary points	251
Conventional antidiabetic drugs or traditional medicines?	215	References	251
Antioxidant and antidiabetic qualities of <i>Centella asiatica</i>	216	25. Glutamine and its antioxidative potentials in diabetes	255
Conclusion	220	SUNG-LING YEH, YAO-MING SHIH AND MING-TSAN LIN	
References	220	List of abbreviations	255
22. Effects of Chrysanthemi Flos against diabetes and its complications related to insulin resistance	223	Introduction to glutamine	255
SUNG-JIN KIM		Cellular functions of glutamine	255
List of abbreviations	223	Antioxidative and antiinflammatory properties of glutamine	256
Diabetes	223	Hyperglycemia-induced oxidative stress and its associated complications	257
Chrysanthemi Flos	224	Effects of glutamine on glucose homeostasis and insulin sensitivity	257
Effects of Chrysanthemi Flos on diabetes and its complications	225	Mechanisms of glutamine in attenuating hyperglycemia-induced oxidative stress and inflammation	259
Effect of Chrysanthemi Flos on insulin resistance	229	Conclusion	262
Effect of Chrysanthemi Flos on other biological activities	229	Summary points	262
Chemical constituents of Chrysanthemi Flos and their activities	230	References	263
Toxic effects of Chrysanthemi Flos	231	26. The antioxidant potential of <i>Lactarius deterrimus</i> in diabetes	265
Conclusion	232	JELENA ARAMBAŠIĆ JOVANOVIĆ, MIRJANA MIHAILOVIĆ, SVETLANA DINIĆ, NEVENA GRDOVIĆ, ALEKSANDRA USKOKOVIĆ, GORAN POZNANOVIĆ AND MELITA VIDAKOVIĆ	
Summary points	232	List of abbreviations	265
References	232	Introduction	265
23. Cinnamic acid as a dietary antioxidant in diabetes treatment	235	Characteristic of the <i>Lactarius</i> species	266
HATICE GÜL ANLAR		Mechanisms and pathways underlying diabetes development	267
List of abbreviations	235	Antioxidant and antiglycation properties of the <i>Lactarius deterrimus</i> extract in vitro	268
Introduction	235	Systemic antioxidant and antiglycation effect of the <i>Lactarius deterrimus</i> extract in vivo	270
Diabetes mellitus and oxidative stress	236	Protective effects of the <i>Lactarius deterrimus</i> extract on pancreatic islets in vivo	271
Chemistry of cinnamic acid	236	Protective effects of the <i>Lactarius deterrimus</i> extract on hepatorenal injury in vivo	271
Dietary source and dietary-intake levels of cinnamic acid	237	Conclusion	271
Pharmacokinetic properties of cinnamic acid	237	Acknowledgments	272
Antioxidant activity of cinnamic acid	237	Summary points	272
Antidiabetic effects of cinnamic acid	238	References	272
Conclusion	239	27. Limonene and ursolic acid in the treatment of diabetes	275
Summary points	240	MERVE BACANLI	
References	242	List of abbreviations	275
24. Cranberry, oxidative stress, inflammatory markers, and insulin sensitivity: a focus on intestinal microbiota	245	Introduction	275
ANA SOFÍA MEDINA-LARQUÉ, YVES DESJARDINS AND HÉLÈNE JACQUES		Diabetes	276
List of abbreviations	245		
Introduction	245		
Cranberry and oxidative stress and systemic inflammation	246		

General information about limonene	277	Overview of <i>Salvia hispanica</i> L.	316
Diabetes and limonene	278	Application to health promotion and disease prevention or improvement	316
General information about ursolic acid	279	Effects of dietary <i>Salvia hispanica</i> L. (Salba) on oxidative stress, adipose tissue dysfunction, dyslipidemia, and insulin resistance	317
Diabetes and ursolic acid	279	Summary points	321
Conclusion	281	References	321
Summary points	281		
References	281		
28. Palm oil: its antioxidant potential in diabetes mellitus	285	32. Spirulina platensis, oxidative stress, and diabetes	325
TOYIN DORCAS ALABI, FOLORUNSO ADEWALE OLABIYI AND OLUWAFEMI OMONIYI OGUNTIBEJU		AREZKI BITAM AND OURIDA AISSAOUI	
List of abbreviations	285	List of abbreviations	325
Introduction	285	Introduction	325
Summary points	289	Diabetes	325
Recommendations and further studies	289	Antioxidants and spirulina	326
References	289	Diabète–SP–oxidative stress	328
		Conclusion	329
		Summary points	329
		References	329
29. Quercetin and antioxidant potential in diabetes	293	Further reading	331
FRANCIS I. ACHIKE AND DHARMANI D. MURUGAN			
List of abbreviations	293	33. Statins, diabetic oxidative stress, and vascular tissue	333
Introduction	293	JONATHAN R. MURROW	
Historic background	293	List of abbreviations	333
Oxidative stress in the etiopathogenesis of diabetes mellitus	294	Introduction	333
The oxygen paradox	294	Oxidative stress and diabetes	333
Sources of oxidative stress	294	Statins: discovery and mechanisms	334
Mitochondria	294	Impact of statins on oxidative stress	335
NADPH oxidases	294	Diabetic macrovascular disease: clinical evidence	337
Xanthine oxidase	295	Diabetic microvascular disease: clinical evidence	338
Polyol pathway	295	Summary and future directions	338
Hexosamine pathway	295	Summary points	338
AGEs and RAGEs	295	References	339
Antioxidants	295		
Conclusion	299	34. Nanoparticle formulation of <i>Syzygium cumini</i>, antioxidants, and diabetes	343
Summary points	299	PAULA E.R. BITENCOURT	
References	299	List of abbreviations	343
Further reading	302	Introduction	343
		Nanotechnology	344
30. Resveratrol in diabetes: benefits against oxidative stress in male reproduction	303	Use of <i>S. cumini</i> and nanoparticles in DM and oxidative stress	346
SANDRA MARIA MIRAGLIA, JOANA NOGUÈRES SIMAS, TALITA BIUDE MENDES AND VANESSA VENDRAMINI		Conclusion	349
List of abbreviations	303	Summary points	349
Introduction	303	References	349
Summary points	312		
References	312	35. Protective role of taurine and structurally related compounds against diabetes-induced oxidative stress	351
		CESAR A. LAU-CAM	
31. <i>Salvia hispanica</i> L. and its therapeutic role in a model of insulin resistance	315	List of abbreviations	351
MARÍA DEL ROSARIO FERREIRA, SILVINA ALVAREZ, PAOLA ILLESCA, MARÍA SOFÍA GIMÉNEZ AND YOLANDA B. LOMBARDO			
List of abbreviations	315		
Introduction	315		

Introduction	351	38. Vitamin D, oxidative stress, and diabetes: crossroads for new therapeutic approaches	385
TAU and diabetes	352	BAHAREH NIKOOYEH, RAZIEH ANARI AND TIRANG R. NEYESTANI	
TAU, diabetes, and the cardiovascular system	352	List of abbreviations	385
TAU, diabetes, and erythrocytes	353	Introduction	385
TAU, diabetes, and the eye	354	Vitamin D	386
TAU, diabetes, and the kidney	355	Oxidative stress in diabetes: development and complications	388
TAU, diabetes, and the liver	356	Vitamin D and diabetes	389
TAU, diabetes, and the nervous system	356	Vitamin D as an antioxidant	390
Conclusions	357	Antioxidant effect of vitamin D in diabetes: direct versus indirect effect	391
Summary points	357	Conclusion	392
References	357	Summary points	393
		References	394
36. Taurine and cardiac oxidative stress in diabetes	361	39. Vitamin E, high-density lipoproteins, and vascular protection in diabetes	397
JOYDEEP DAS, SUMIT GHOSH AND PARAMES C. SIL		TINA COSTACOU, JOSHUA B. WIENER, ELLIOT M. BERINSTEIN AND ANDREW P. LEVY	
List of abbreviations	361	List of abbreviations	397
Introduction	361	Introduction	397
Diabetes-induced oxidative stress	362	The haptoglobin protein	398
The role of mitochondria in ROS production	362	High-density lipoproteins	400
Advanced glycation end-products (AGE)-mediated ROS production	363	Haptoglobin and high-density lipoprotein	401
The role of NADPH oxidase in ROS production	363	Vascular protection by vitamin E	401
The role of CaMKII in ROS production	363	Summary/future directions/conclusions	403
The role of fatty acids in ROS production	363	Summary points	404
The role of the polyol pathway in ROS production	363	References	404
The role of Nrf2 in ROS production	363		
The role of xanthine oxidase in ROS production	363		
The role of increased hexosamine flux in ROS production	364		
The role of PKC activation in ROS production	364		
The role of angiotensin II activation in ROS production	365		
Endogenous antioxidant mechanisms	365		
The beneficial role of taurine	365		
Depletion of taurine in the myocardium due to diabetic cardiomyopathy	365		
Mechanisms of the antihyperglycemic action of taurine	366		
The antioxidant mechanism of taurine against cardiac oxidative stress under diabetic conditions	367		
Combinatorial therapies involving taurine for the treatment of cardiac oxidative stress	370		
Summary points	370		
References	370		
37. Vitamins, antioxidants, and type 2 diabetes	373	Section III	
FERNANDA S. TONIN, HELENA H. BORBA, ASTRID WIENS, FERNANDO FERNANDEZ-LLIMOS AND ROBERTO PONTAROLO		Techniques and resources	
List of abbreviations	373		
Introduction	373	40. Superoxide dismutase as a measure of antioxidant status and its application to diabetes	409
Free radicals, oxidative stress, and diabetes	374	FELIX OMORUYI, JEAN SPARKS, DEWAYNE STENNETT AND LOWELL DILWORTH	
Antioxidants and their role in type 2 diabetes	375	Introduction	409
Vitamins and antioxidant mechanisms	375	Oxidative stress in diabetes	411
Vitamin supplementation in type 2 diabetes: clinical evidence	378	Complications associated with diabetes	411
Conclusions	378	Oxidative damage	413
Summary points	381	Oxidative defense system	413
References	382	Superoxide dismutase	414
		Superoxide dismutase activity determination	414
		Pyrogallol autoxidation method	414
		Calculation of SOD activity	414
		In-Gel assay	414
		Early detection and treatment of diabetes	414
		Summary points	416
		References	416

41. Recommended resources for diabetes, oxidative stress, and dietary antioxidants	419	Acknowledgements	421
RAJKUMAR RAJENDRAM, VINOOD B. PATEL AND VICTOR R. PREEDY		Summary points	421
Introduction	419	References	422
Resources	419	Index	423

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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.

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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

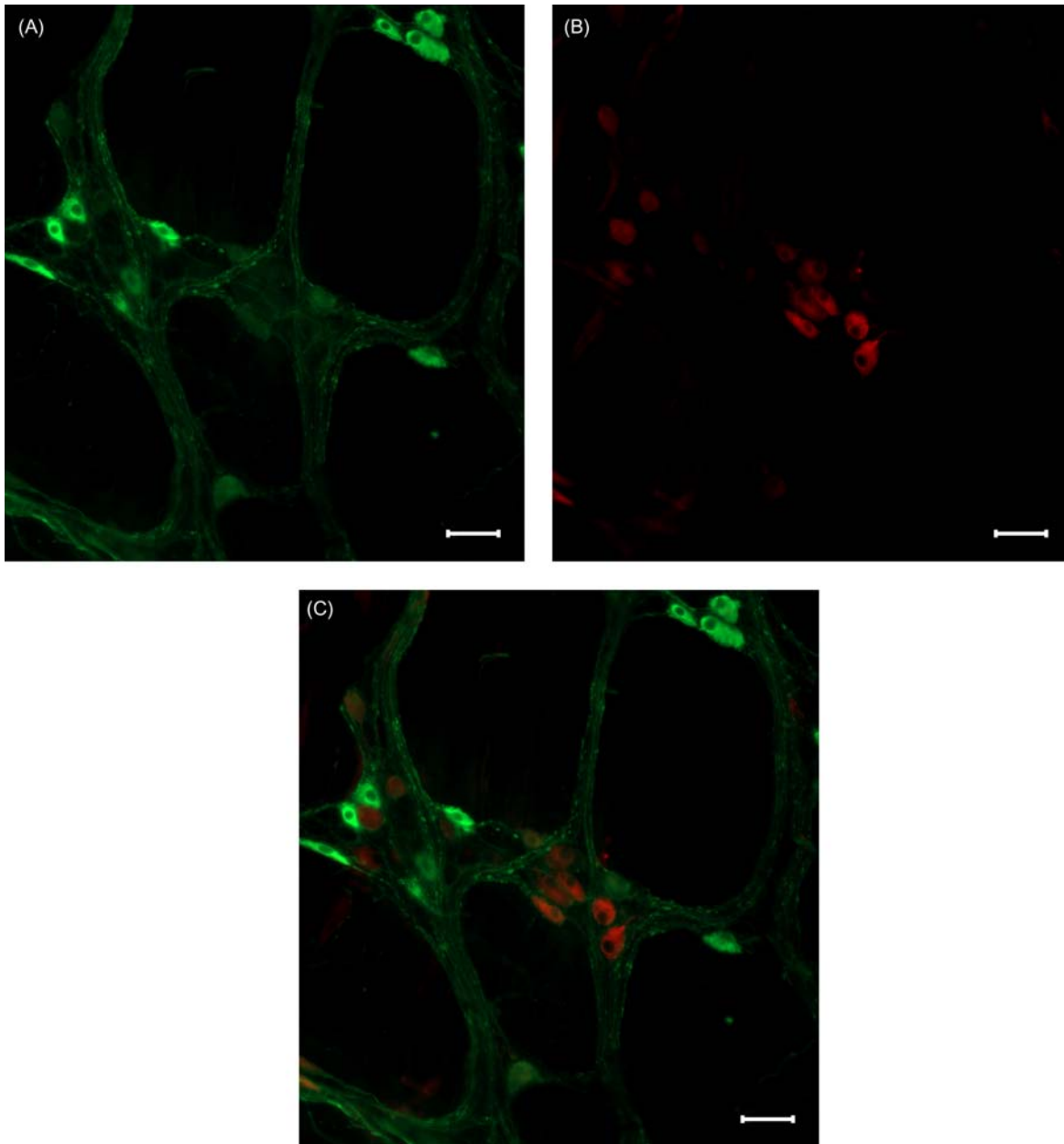


FIGURE 3.1 Photomicrographs of neuronal nitric oxide synthase (A) neurofilament 200 (B) immunostained myenteric neurons in a whole-mount preparation from the colon of a control rat. Figure C shows the merged pictures. Scale bars: 50 μm .

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 Celtek 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 earlier¹⁵ and differs from segment to segment along the gastrointestinal tract.¹³

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

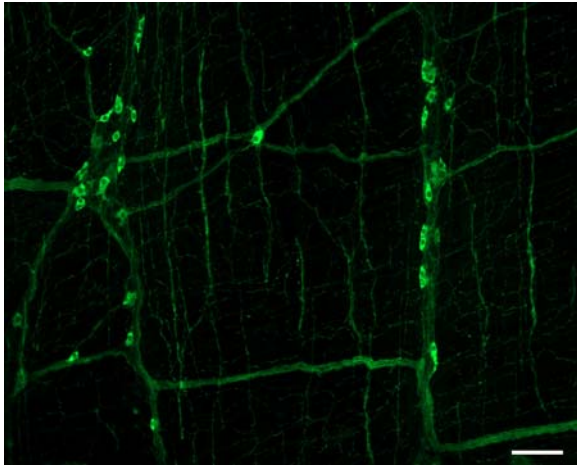


FIGURE 3.2 Photomicrograph of neuronal nitric oxide synthase-immunostained myenteric neurons in a whole-mount preparation from the duodenum of a control rat. The number of nitrergic neurons is notable in the myenteric plexus. The main function of the nitrergic myenteric neurons is the regulation of the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

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 ileum³⁰ 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 showed³² 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).

Plexus	Marker	Gut Segment		
		Duodenum	Ileum	Colon
SP ³¹	nNOS	∅	↑2x	↑3x
	HuC/D	∅	∅	∅
MP ¹³	HuC/D	∅	↓	↓
	nNOS	↓	↓	↓

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

Plexus	Marker	Gut Segment		
		Duodenum	Ileum	Colon
SP ³¹	HO2	∅	∅	∅
	HO1	∅	∅	∅
MP ¹⁴	HO1	∅	↑7x	↑1.5x
	HO2	∅	↑2x	∅
	HO1-nNOS	∅	↑7x	↑2x
	HO2-nNOS	∅	↑6x	∅

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 nitrenergic 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 nitrenergic 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 Lincoln⁵⁰ 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 nitrenergic 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 diabetics¹⁴ 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 ileum¹⁴ compared to controls. This data supports that many of the nitrenergic 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 ileum²⁸ 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 nitrenergic and the total neuronal number decreased significantly.^{13,35}

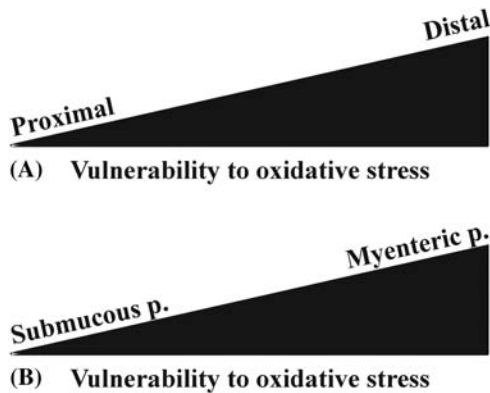


FIGURE 3.5 Vulnerability of the gut and enteric plexuses to diabetes-related oxidative stress. The distal part of the gut is more vulnerable than the proximal to diabetic oxidative stress (A). While myenteric neurons are more susceptible to diabetic damage, the submucous ganglia have greater resistance 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, presumably 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 nitregeric neurons are less damaged ¹⁴ Higher representation of order <i>Lactobacillales</i> ²⁸
Lipid peroxidation [↑] ^{48,49} Protein oxidation [↑] ⁴⁹ <i>Klebsiella</i> invasion ²⁸ Microbiota dysbiosis ^{28,53}	Ileum	Superoxide dismutase, catalase, glutathione [↓] ⁴⁸ Greatest induction of HO1 and HO2 expression in nitregeric neurons ¹⁴
Peroxyntirite [↑] ³⁶ Microbiota dysbiosis ²⁸	Colon	Superoxide dismutase [↓] ³⁶ Catalase, glutathione [∅] ³⁶ Metallothionein 1 mRNA [↑] ³⁶ Induction of HO system in nitregeric 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

- Nitregeric 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.
- Nitregeric 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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