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Nutritional Antioxidant Therapies: Treatments and Perspectives

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Preface

Before the creation of any life forms on earth, gas molecules were ubiquitous in the atmosphere. Among these molecules, oxygen became a key chemical element for biological organisms to respire and to produce the necessary energy for various activities of life. Existing in an environment where oxygen can have untoward risks seems paradoxical, since we breathe oxygen not only to survive but also to endure the consequences of the production of free radical and non-radical molecules collectively referred to as reactive oxygen species (ROS). Because ROS are generated as natural by-products of aerobic respiration and metabolism to become ubiquitous molecules in all biological system, every aspect in the life of aerobic organisms is affected by this paradox. Mitochondria are a major source of ROS production within and out of the cell. Our bodies produce ROS as part of normal physiological functions such as intracellular signaling and mediating some immune responses.

However, we are also exposed to multitude of human-made chemicals, such as pesticides, heavy metals, endocrine disruptors, ambient air particulate matter, and industrial solvents. Added to this ROS is accumulation resulting from unhealthy lifestyle behaviors, mainly poor diet habits, physical inactivity, tobacco smoking, alcohol consumption, and drug abuse.

Overproduction of ROS induces oxidative stress, a state where increased generation of ROS overwhelms antioxidant protection, leading to oxidative damage of cellular macromolecules, including proteins, lipids, and nucleic acids. The consequences of this chemical oxidative damage include loss of enzyme activity, cell membrane alterations and damage, DNA lesions, and mutagenesis. During the past three decades, oxidative stress has been linked to prenatal and postnatal developmental disorders; to adult noncommunicable diseases, including diabetes, cancer, and reproductive, gastrointestinal, hepatic, renal, pulmonary, cardiovascular, and neurological diseases; and to the aging process. There is also a possibility that the risks of several noncommunicable and oxidative stress chronic diseases may have prenatal origins. Antioxidant nutritional therapies could therefore be highly beneficial to embryonic and fetal development, neonatal growth and health, maintenance of good adult health, and potentially healthy aging.

Epidemiological and clinical studies have linked undernutrition, malnutrition, and unhealthy lifestyle behaviors to multiple diseases and premature aging in association with oxidative stress due to insufficient antioxidant protections and ROS detoxification. In addition, industrial and agricultural activities contribute to the release of large quantities of chemical pollutants in the environment and have already resulted in widespread soil and water contamination. Exposure to these pollutants is inevitable as it occurs through the consumption of contaminated food and water as well as by air inhalation. Therefore, the ability to feed properly and to have healthy lifestyle behaviors is the greatest concern of humans, since we rely on our ability to generate safe nutrients, high-quality products, and healthy plant-based diets while minimizing adverse environmental impacts on health. There is increasing interest in seeking health remedies, leading to the need by many to resort to plant-based therapies since dietary organic and inorganic substances have significant antioxidant levels. Plant antioxidants have increased in popularity as evidenced by the widespread adherence to traditional medicine and its use as an adjuvant in conventional health care.

The purpose of our book was to assemble a series of expert reviews on the therapeutic potential of plant antioxidants as effective and reliable natural compounds to combat oxidative stress omnipresent during the life of organisms in utero, postnatal development, adulthood, and aging. This book provides an overview of the use of plant-based antioxidant therapies in health promotion and disease prevention and treatment.

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Acknowledgments

The editors identified the need of a comprehensive reference book focusing on current knowledge of the therapeutic potential of plant antioxidants as a guide to reviving guidelines and nutritional health-promoting recommendations. This collection of reviews also aims to increase awareness of healthy nutrition, dietary habits, and diets.

This book is a collection of 20 peer-reviewed chapters, each of which is written by international content experts and collectively covers complementary themes related to the role of nutritional antioxidant therapies in health promotion and disease prevention. Assembling a multiauthored specialized book is usually a major challenge for authors and the editors, but in this case it has been an enjoyable and fulfilling experience largely due to the enthusiasm of the authors, reviewers, and publisher.

It is with immense pleasure to gratefully acknowledge and congratulate all the authors and reviewers who helped us to assemble this book. In addition, we warmly thank our colleagues at Springer-Nature for their keen interest and commitment to the publication of this new book.

Lastly, we would like to thank our mentors, colleagues, and family members for their continued support and guidance.

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List of Abbreviations

AA	Arachidonic acid
ABTS	2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid
ACC	Acetyl-coenzyme A carboxylase
ACC	CoA carboxylase
ACE	Angiotensin-converting enzymes
AD	Alzheimer's disease
ADMA	Asymmetric dimethylarginine
ADP	Adenosine diphosphate
AFB	Aflatoxin B
AFR	Ascorbate free radical
AGE	Advanced glycation end products
AhR	Aryl hydrocarbon receptor
Akt	Protein kinase B
ALA	Alpha-lipoic acid
ALD	Alcoholic liver disease
ALS	Amyotrophic lateral sclerosis
ALT	Alanine transaminase
AMP	Adenosine monophosphate
AMPK	5′ Adenosine monophosphate-activated protein kinase
AngII	Angiotensin II
AOPP	Advanced oxidation protein products
AP-1	Activator protein-1
aPKC	Atypical protein kinase C
APOE	Apolipoprotein
APP	Amyloid precursor protein
APX	Ascorbate peroxidase
AQP	Aquaporins
AR	Aldose reductase
ARE	Antioxidant response elements
ARNT	Aryl hydrocarbon receptor nuclear translocator
ARs	Androgen receptors

ART	Assisted reproductive technologies
As	Arsenic
$Asc^{\bullet -}$	Ascorbate free radical
$Asc2^{-}$	Ascorbate radical
$AscH^{-}$	Ascorbate anions
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
Aβ	Amyloid beta
Aβ1-40	Amyloid β peptide-40
BACE-1	Beta-site APP cleaving enzyme-1
BAL	British anti-Lewisite
BCRP	Breast cancer resistance protein
BDNF	Brain-derived neurotrophic factor
Be	Beryllium
BH4	Heme tetrahydrobiopterin
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BP	Blood pressure
BPA	Bisphenol A
BUB1	Budding uninhibited by benzimidazoles 1
C/EBPα	CCAAT-enhancer-binding protein alpha
C/EBPβ	CCAAT-enhancer-binding protein β
CARET	Beta-carotene and retinol efficacy trial
CAT	Catalase
CBS	Cystathionine b synthase
CCNB2	Cyclin B2
Cd	Cadmium
CD	Crohn's disease
CDC20	Cell-division cycle protein 20
CDDP	Cis-diammine dichloroplatinum
CDKs	Cyclin-dependent kinases
CDO	Cysteine dioxygenase
CGs	Catechin gallates
CHD	Coronary heart disease
CγL	Cystathionine gamma lyase
CNS	Central nervous system
CO	Carbon monoxide
Co	Cobalt
CO_2	Carbon dioxide
CoQ10	Coenzyme Q10
COX	Cyclooxygenase
CPP	Central precocious puberty
CPT-1	Carnitine palmitoyltransferase-1
Cr	Chromium
CREB	cAMP response element-binding protein

CSD	Cysteine sulfinate decarboxylase
CSF	Cerebrospinal fluid
Cu	Copper
CVDs	Cardiovascular diseases
Cy-3-glu	Cyanidin-3-glucoside
CYP	Cytochromes P450
CYP7A1	Cholesterol 7α-hydroxylase
CysDA	5-S-Vysteinyl-dopamine
d-ROMs	Reactive oxygen metabolites test
DAG	Diacylglycerol
DASH	Dietary approaches to stop hypertension
DDAH	Dimethylarginine dimethylamino hydrolase
DDE	2,2'-bis(4-Chlorophenyl)-1,1-dichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DE	Diesel exhaust
DHAR	Dehydroascorbate reductase
DHLA	Dihydrolipoic acid
DMB	Demethyleneberberine
DMES	Diabetic Macular Edema Study
DMPS	2,3-Dimercapto-1-propanesulfonic acid
DMSA	Dimercaptosuccinic acid or succimer
DMT1	Divalent metal transporter 1
DNA	Deoxyribonucleic acid
DPP4	Dipeptidyl peptidase-4
DPPH	2,2-Diphenyl-1-picrylhydrazyl
E2F5	Transcription factor E2F5
EC	Epicatechin
ECG	Epicatechin gallate
EDHF	Endothelium-derived hyperpolarizing factor
EDTA	Ethylenediaminetetraacetic acid
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
EGF	Epidermal growth factor
eNOS	Endothelial nitric oxide synthase
ERK	Extracellular signal-regulated kinase
ERK1/2	Extracellular signal-regulated protein kinases 1 and 2
ERKs	Extracellular signal-regulated protein kinases
ERs	Estrogen receptors
ET-1	Endothelin-1
F2-IsoPs	F2-isoprostanes
FAD	Flavin adenine dinucleotide
FAS	Fatty acid synthase
FASTT	Fast track and standard treatment trial
Fe	Iron
FGFR2	Fibroblast growth factor receptor 2

FMN	Flavin mononucleotide
FOXO1	Forkhead box protein O1
FRAP	Ferric reducing antioxidant power
FRAP	Fluorescence recovery after photobleaching
FSH	Follicle-stimulating hormone
FSH	Follicle-stimulating hormone
FTC	Ferric thiocyanate
g-TE	γ-Tocotrienol
G6Pase	Glucose-6-phosphatase
G6PD	Glucose-6-phosphate dehydrogenase
GC	Gas chromatography
GCL	Glutamate cysteine ligase
GCS	Glutamylcysteine synthetase
GHES	Glucosyl hesperidin
GLP-1	Glucagon-like peptide-1
Glu	Glucose transporter
GLUT	Glucose transporter
GLUT4	Glucose transporter 4
GNT	Gluconasturtiin
GPAT-1	Glycerol-3-phosphate acyltransferase-1
G6PD	Glucose-6-phosphate dehydrogenase
GPR30	G-protein-coupled receptor 30
GPX	Glutathione peroxidase
GR	Glutathione reductase
$GS^{\bullet}$	Thiyl radicals
GSH	Glutathione
GSS	Glutathione synthetase
GSSG	Glutathione disulfide
GST M1	Glutathione S-transferase mu 1
GST	Glutathione S-transferase
GTC	Green tea catechins
H_2O_2	Hydrogen peroxide
HA	Heterocyclic amines
HbA1c	Hemoglobin A1c
HbA1c	Hemoglobin A1c
HBV	Hepatitis B
HCC	Hepatocellular carcinoma
HCl	Hydrochloric acid
HCV	Hepatitis C virus
Hcy	Homocysteine
HD	Huntington's disease
HDL-C	High-density lipoprotein cholesterol
4-HNE	4-Hydroxynonenal
HES	Hesperidin
HETEs	Hydroxyeicosatetraenoic acids

Hg	Mercury
HIF-1α	Hypoxia-inducible factor 1α
HLE-B3	Human lens epithelial cell line
HMG-CoA	3-Hydroxy-3-methylglutaryl-coenzyme A
HNE	4-Hydroxynonenal
HO-1	Heme oxygenase-1
HOCl	Hypochlorous acid
HOMA-IR	Homeostatic model assessment of insulin resistance
HOPE	Heart Outcomes Prevention Evaluation
HpCDD	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin
HpETEs	Hydroperoxyeicosatetraenoic acids
hs-CRP	High-sensitivity C-reactive protein
HSL	Hormone-sensitive lipase
HSP	Heat-shock protein
IBD	Inflammatory bowel disease
ICAM-1	Intercellular adhesion molecule 1
ICDH	Isocitrate dehydrogenases
ICSI	Intracytoplasmic sperm injection
IDL	Intermediate density lipoprotein
IFN-γ	Interferon gamma
IFOAM	International Federation of Organic Agriculture Movements
IHD	Ischemic heart disease
IKK	IκB kinase
IL-1α	Interleukin 1 alpha
IL-1β	Interleukin 1 beta
IL-2	Interleukin 2
IL-6	Interleukin 6
IL-8	Interleukin 8
iNOS	Inducible nitric oxide synthase
IR	Ionizing radiation
IRS-1	Insulin receptor substrate 1
IRS-2	Insulin receptor substrate 2
ITCs	Isothiocyanates
IUGR	Intrauterine growth restriction
IVF	In vitro fertilization
IVF/ET	In vitro fertilization and embryo transfer
JNK	c-Jun N-terminal kinase
Keap1	Kelch-like ECH-associated protein 1
LDL-C	Low-density lipoprotein cholesterol
LDL	Low-density lipoprotein
LDLox	Oxidized low-density lipoprotein
LH	Luteinizing hormone
LOO•	Lipid peroxyl radicals
LOX	Lipoxygenase
LPH	Lactase-phlorizin hydrolase

LPS	Lipopolysaccharide
LTB4	Leukotriene B4
LTC4	Leukotriene C4
LTs	Leukotrienes
MAG	Monoglyceride
MAPKs	Mitogen-activated protein kinases
MCDD	Methionine and choline-deficient diet
MCI	Mild cognitive impairment
MCP1	Monocyte chemoattractant protein-1
MD	Mediterranean diet
MDA	Malondialdehyde
MEOS	Microsomal ethanol oxidizing system
MMPs	Matrix metalloproteinases
MPO	Myeloperoxidase
MPP+	1-Methyl-4-phenylpyridinium
MPT	Mitochondrial permeability transition
MRL	Maximum residue limits
MS	Mass spectrometry
MS	Multiple sclerosis
MTA	Mitochondrial-targeted antioxidant
mtDNA	Mitochondrial DNA
MTF-1	Metal-responsive transcription factor-1
MYBL2	MYB proto-oncogene-like 2
NAC	N-Acetyl-cysteine
NAD	Nicotinamide adenine dinucleotide
NADH	Oxidized nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NCDs	Noncommunicable diseases
NF-κB	Nuclear factor-kappa B
NHANES	National Health and Nutrition Examination Survey
Ni	Nickel
NIH	National Institutes of Health
NLCS	Netherlands Cohort Study
NMR	Nuclear magnetic resonance
NMU	N-Nitrosomethylurea
nNOS	Neuronal nitric oxide synthase
$NO^{\bullet}$	Nitric oxide
NO_2-Tyr	3-Nitro-tyrosine
NO_2	Nitrogen dioxide
NOS	Nitric oxide synthase
NQO1	Nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase
Nrf2	Nuclear factor (erythroid-derived 2)-like 2

O_2	Oxygen
O_3	Ozone
$^1O_2$	Singlet oxygen
$^{\bullet}O_2^-$	Superoxide radical
OAT	Oligoasthenoteratozoospermia
OC	Organochlorine
OCDD	1,2,3,4,6,7,8,9-Osctachlorodibenzo-p-dioxin
OCTNs	Organic cation transporters
OECD	Organization for Economic Cooperation and Development
$^{\bullet}OH$	Hydroxyl radical
6-OHDA	6-Hydroxydopamine
8-OHdG	8-Hydroxy-2′-deoxyguanosine
8-OHG	8-Hydroxyguanosine
4-ONE	4-Oxo-trans-2-nonenal
$ONOO^-$	Peroxynitrite anion
ORAC	Oxygen radical absorbance capacity
8-oxodG	8-Hydroxy-2′-deoxyguanosine
P13K	Phosphatidylinositol 3-kinase
p38MAPK	p38 mitogen-activated protein kinase
PAF	Platelet-activating factor
PAH	Polycyclic aromatic hydrocarbons
PAI-1	Plasminogen activator fibrinogen inhibitor-1
PARP	Poly (ADP-ribose) polymerase
Pb	Lead
pBD-1	Porcine beta-defensin 1
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzodioxins
PCDFs	Polychlorinated dibenzofurans
PCNA	Proliferating cell nuclear antigen
PCOS	Polycystic ovarian syndrome
PD	Parkinson's disease
PDGF	Platelet-derived growth factor
PDX-1	Pancreas duodenum homobox-1
PEITC	Phenylethyl isothiocyanate
PEPCK	Phosphoenolpyruvate carboxykinase
PERK	Protein kinase-like endoplasmic reticulum kinase
PG	Propyl gallate
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PGE2	Prostaglandin E2
PGI2	Prostacyclin
PGI2	Prostaglandin I2
PI3K	Phosphatidylinositol 3-kinase
PKA	Protein kinase A

PKB	Protein kinase B
PKC	Protein kinase C
PLA2	Phospholipase A2
PM	Particulate matter
PMRS	Plasma membrane redox system
PMS	Premenstrual syndrome
POPs	Persistent organic pollutants
PPARγ	Peroxisome proliferator-activated receptor gamma
PRX	Peroxiredoxin
PSA	Prostate-specific antigen
PTP-1B	Tyrosine protein phosphatase 1B
PUFAs	Polyunsaturated fatty acids
QR	Quinone reductase
RDA	Recommended daily allowance
RNS	Reactive nitrogen species
$RO2^{\bullet}$	Peroxyl
$RO2^{\bullet}$	Peroxyl radical
RO5	Lipinski's five rules
ROH	Alcohol
ROOH	Hydroperoxides
ROS	Reactive oxygen species
SAPKs	Stress-activated protein kinases
sAβPPα	Amyloid precursor protein-α
Se-OH	Selenoles
SGA	Small for gestational age
SGLT	Sodium-dependent glucose co-transporter
SGLT2	Sodium-glucose co-transporter 2
sHBG	Sex hormone-binding globulin
SIRT	Sirtuin
SLC23A1	Sodium-dependent vitamin C transporters
SO_2	Sulfur dioxide
SOCS	Cytokine signaling proteins
SOD	Superoxide dismutase
SPPB	Short physical performance battery
SREBP-1c	Sterol regulatory element-binding protein 1c
STAT	Signal transducer and activator of transcription
sVCAM-1	Soluble vascular cell adhesion molecule-1
SVCT	Sodium-ascorbate co-transporter
T2D	Type 2 diabetes
T2DM	Type 2 diabetes mellitus
TAC	Total antioxidant capacity
TAS	Total antioxidant status
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid-reducing substances
TBHQ	Tertiarybutyl hydroquinone

TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TCS	Triclosan
TEAC	Trolox equivalent antioxidant capacity
TLC	Thin-layer chromatography
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha
TOS	Total oxidant status
TPP+	Tetraphenylphosphonium cation
TRAP	Total radical-trapping parameter
TRAP	Total reactive antioxidant potential
TRX	Thioredoxins
TRXR	Thioredoxin reductases
α-TTP	α-Tocopherol transport protein
TXA2	Thromboxane A2
Tyr10	Tyrosine 10
UC	Ulcerative colitis
UCP-2	Uncoupling protein 2
UDP-GT	UDP-glucuronosyltransferase
UL	Tolerable upper intake
UN	United Nations
uPA	Urokinase plasminogen activator
US	United States
USDA	United States Department of Agriculture
UV	Ultraviolet
V	Vanadium
VCAM-1	Vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factor
VLDL	Very-low-density lipoprotein
WASH 1	Warfarin/Aspirin Study in Heart failure
WHO	World Health Organization
XO	Xanthine oxidase
XOR	Xanthine oxidoreductase
XRE	Xenobiotic response element
Zn	Zinc

About the Editors

Editor's Biography

Kaïs Hussain Al-Gubory is senior researcher and engineer at the National Institute for Agricultural Research in France. After receiving his undergraduate degree in zoology, microbiology, and biochemistry at the University of Baghdad (Iraq) with a First-Class Honours degree, he worked as an assistant lecturer at the same university. Supports from the Ministry of Higher Education and Scientific Research (Iraq), the National Institute for Agricultural Research, and the Medical Research Foundation (France) encouraged him to pursue a postgraduate education, with completion of a DEA degree (Diplôme d'Etudes Approfondie), a PhD (Doctorat de Troisième Cycle), and a higher education degree of PhD (Thèse de Doctorat d'Etat ès Sciences Naturelles) at the University of Rennes (France). Al-Gubory's career in research has focused on reproductive physiology where he studies the interactions between reproductive hormones and their target organs. He was the first to isolate and purify the superoxide dismutase from sheep corpus luteum of pregnancy and demonstrated the role played by this antioxidant enzyme in the control of ovarian-pituitary endocrine functions. He has made significant contributions to the understanding of the role of antioxidants in reproduction and prenatal development. He was actively involved in the development of the fibered confocal fluorescence microscopy (Cell-viZio) for in vivo imaging of tissues and organs. This breakthrough in live cell imaging established a foundation for the consequent application of this technology in biomedical imaging and clinical studies. Dr. Al-Gubory's current research interests include examining the impact of environmental factors on early life development and the beneficial effects of plant antioxidant compounds in prenatal development, with the aim of health

promotion and prevention of early life programming diseases via modulation of oxidative stress. Dr. Al-Gubory has over 90 peer-reviewed publications, book chapters, communications, and reports.

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Ismail Laher is professor of pharmacology in the Department of Pharmacology and Therapeutics in the Faculty of Medicine at the University of British Columbia (UBC) in Vancouver, Canada. He is also a visiting professor in the Department of Pharmacology in the Faculty of Medicine at the University of Vermont (USA). After receiving his undergraduate degree in pharmacology at Chelsea College (University of London, UK), he continued with graduate studies in cardiovascular pharmacology at Memorial University and UBC (Canada). Following several years at the University of Vermont, he returned to UBC. Ismail Laher's research is in understanding resistance artery function in health and disease. He has edited a five-volume collection *Systems Biology of Free Radicals and Antioxidants* (Springer-Verlag, 2014). The major research interest/expertise of Professor Laher includes studies of the regulation of vascular function by oxidative stress in an animal model of sleep apnea and of the effects of exercise in modulating oxidative stress in an animal model of type 2 diabetes. Professor Laher has published over 150 peer-reviewed articles and several book chapters on autonomic pharmacology, myogenic regulation of small arteries, and functional characterization of vascular function in animal models of disease (diabetes, hypertension, obesity, sleep apnea, spinal cord injury). He also serves on the editorial board of several journals.

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Chapter 1
Introducing Chapter: Phytochemicals, Antioxidant Therapy, Opportunities and Challenges

Kaïs Hussain Al-Gubory

Abstract The use of phytochemicals for health promotion and disease prevention and treatment has gained increased interest worldwide particularly because of their availability, low cost and minimal side effects. Phytochemicals have a wide range of biological properties such as antioxidative, anti-proliferative, anti-inflammatory and antiobesogenic. They are believed to reduce oxidative stress in the body and the risk of noncommunicable diseases (NCDs). The interest in phytotherapy lies in the use of whole or parts of plants, or plant-derived extracts, containing different antioxidants, which function synergistically and in combination with each other to reduce oxidative stress. There is also an increasing tendency to recommend regular intake of plant-based diets to forestall oxidative stress-induced human diseases. Although the primary strategy of disease prevention should focus on health promotion, there are people in many regions all over the world still dying of preventable and curable diet-related and/or lifestyle-related NCDs, which are often associated with undernutrition, malnutrition, unhealthy lifestyle behaviours and exposure to human-made pollutants. Although multiple strategies are needed to ensure and improve a healthy life, such as changes in dietary habits, food production and consumption, and lifestyle behaviours, physicians and many people are unaware of the benefits of good nutrition based on plant antioxidants that could be used in NCD prevention. This is a challenging area of interest that presents important promise for the near future. Last but not least, feeding a growing world population; fighting hunger, undernourishment and malnutrition; preserving the environment and biodiversity; and producing healthy foods that promote health and prevent diseases are the major global challenges we face today.

Keywords Plants • Phytochemicals • Plant-based diet • Antioxidant therapy • Oxidative stress • Noncommunicable diseases

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1.1 Plants and History of Traditional Medicine

The knowledge of medicinal plants is as old as human early civilization. Plant and animal domestication and the emergence of agriculture in the Fertile Crescent (Upper Mesopotamia), which took place under favourable environmental conditions (Araus et al. 2014), inspired and propelled communities to promote the use of phytotherapy. Indeed, archaeological and textual evidence have revealed that the usage of plants and their derivatives to treat human pathology has contributed to our understanding of the science of medicine and healing that was born in the cradle of flourishing ancient civilizations, Mesopotamia (Borchardt 2002; Kelly 2009), the land (current Iraq) between the Tigris and Euphrates rivers. In more recent time, people around the world have searched and used plants to cure many diseases even before the discovery of a broad range of bioactive phytochemical compounds including antioxidants (Bernhoft 2010; Chikezie et al. 2015).

The number of described flowering plants in the world varies from 223,300 to 315,903 (Chapman 2009), and many of them are valuable sources of therapeutic molecules "medicinal plants" that represent an important reserve for the identification of novel drug leads (Atanasov et al. 2015). Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant-, animal- and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to prevent and treat illnesses or maintain well-being (WHO 2008). The key facts reported by World Health Organization (WHO 2008) regarding the use, practices and expenditure of traditional medicine and complementary/alternative medicine in many developed, developing and underdeveloping countries are listed below:

- In China, traditional herbal preparations account for 30–50% of the total medicinal consumption.
- In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home.
- WHO estimates that in several African countries, traditional birth attendants assist in the majority of births.
- In Europe, North America and other industrialized regions, over 50% of the population have used complementary or alternative medicine at least once.
- In San Francisco, London and South Africa, 75% of people living with HIV/AIDS use traditional medicine and complementary/alternative medicine.
- Seventy percent of the population in Canada have used complementary medicine at least once.
- In Germany, 90% of the population have used a natural remedy at some point in their life.
- Between 1995 and 2000, the number of doctors who had undergone special training in natural remedy medicine had almost doubled to 10,800.
- In the United States, 158 million of the adult population use complementary medicines, and according to the US Commission for Alternative and

Complementary Medicines, US$ 17 billion was spent on traditional remedies in 2000.
- In the United Kingdom, annual expenditure on alternative medicine is US$ 230 million.
- The global market for herbal medicines currently stands at over US$ 60 billion annually and is growing steadily.

Currently, there is a renewed and growing interest in plant traditional medicine, mainly in the African continent and Indian subcontinent. An example of traditional medicine, which dates back to about 5000 years ago, is the Ayurveda (science of life) in use today for individuals and communities in the Indian subcontinent. Ayurveda is an easily accessible and natural medical system, which possesses an established body of written knowledge. Ayurveda was recognized by the government of India as a complete health system comparable to allopathic medicine, as well as by the National Institutes of Health (NIH) as a complementary and alternative medicine (Mishra 2004). Ayurveda has a considerable scientific base and therapeutic potential that can be used alone or in addition to conventional healthcare (Mishra 2004). The basic concepts reported in Mishra's book (Mishra 2004) regarding the use of Ayurvedic medicine in developed, developing and underdeveloping countries are listed below:

- Whereas conventional medicine is primarily oriented towards the treatment of disease, Ayurvedic medicine is oriented towards prevention, health maintenance and treatment.
- In conventional medicine, drugs are developed based on the concept that the elimination of specific causes of a disease, such as microorganisms, will cure a disease.
- The belief in Ayurvedic medicine is that a disease is the product of an imbalance in the body and mental elements that reduce the body's resistance to diseases.
- If the imbalance is corrected and the body's defence mechanisms are strengthened by herbal formulas, lifestyle changes and diet, then the body will resist a disease with a goal of eliminating it.
- Herbal and herb mineral products regularly used in Ayurveda are believed to strengthen the body's defences.
- Scientific evidence is gradually developing in support of the Ayurvedic concept.

The antioxidant, anti-inflammatory, anti-atherosclerotic, anti-proliferative, anti-carcinogenic, anti-diabetic or neuroprotective properties of Ayurveda have been extensively studied in humans and animal models, both in vitro and in vivo (Thabrew et al. 2001; Auddy et al. 2003; Chainani-Wu 2003; Kaur et al. 2004; Govindarajan et al. 2005; Reddy et al. 2005; Dhanasekaran et al. 2007; Jurenka 2009; Baliga 2010; Choedon et al. 2010; Krishnaveni and Mirunalini 2010; Mishra et al. 2011; Chahar et al. 2012; Bag et al. 2013; Cock 2015; Riya et al. 2015; Durg et al. 2015; Baliga et al. 2016; Patwardhan and Bhatt 2016; Keshari et al. 2016; Qadir et al. 2016;

Meghwani et al. 2017). A recent report indicates that traditional medicine based on the use of plants can be applied in health promotion, as well as adjuvant therapy to modern medicine in India (Oyebode et al. 2016).

The United Nations (UN) reported that the current world population has exceeded seven billion, and the continent of Africa is the second most populous (UN 2016). Over the past 36 years, Africa's population increased by almost 722 million, from an estimated 478 million in 1980 to 1.2 billion in 2016, and is projected to reach nearly 2.92 billion in 2063 (UN 2016). Such a demographic situation threatens food and health security. In addition, poverty forces people to undernourishment and/or unhealthy diets, which are major risk factors for non-communicable diseases (NCDs). The galloping demography, poverty, undernutrition and lack of basic healthcare are among the reasons why plants and phytotherapy are used and needed by the majority of the population in Africa for healthcare. Traditional medicine using plants became an important medical system for health promotion and disease treatment in Africa (Abdullahi 2011; Moyo et al. 2015; Innocent 2016). Like the Ayurvedic medicine, the African traditional healthcare system also offers a wide variety of phytochemicals proven as antioxidants with anti-inflammatory, anti-proliferative, anti-mutagenic, anti-carcinogenic, anti-hyperglycaemic or anti-diabetic properties (Gyamfi et al. 1999; Ohtani et al. 2000; Okoli and Akah 2000; Gyamfi and Aniya 2002; Osadebe and Okoye 2003; Ojewole 2006, 2007, 2008; Verschaeve and Van Staden 2008; Fawole et al. 2010; Suleiman et al. 2010; Adeyemi et al. 2011; Tamiru et al. 2012; Bothon et al. 2013; Ishola et al. 2014; Mohammed et al. 2014; Ochwang'i et al. 2014; Birru et al. 2015; Sulyman et al. 2016; Zingue et al. 2016; Amuri et al. 2017). In Africa, more than 5400 plant species are used in traditional medicine for the prevention and treatment of various pathologies (Van Wyk 2015).

1.2 Plant Antioxidants

The kingdom Plantae comprises eukaryotic and multicellular organisms, mostly autotrophic. Thanks to sunlight, plants can make their own food via photosynthesis in the chloroplast. Plant cells function to convert the atmospheric carbon dioxide into carbohydrates, fats, proteins and various molecules, which are indispensable for survival of all living organisms. Plants also provide the oxygen required for all aerobic organisms. Plant mitochondria and chloroplasts, functionally linked to cellular respiration and photosynthesis, respectively, are originally derived from endosymbiotic bacteria (Raven 1970; Andersson et al. 1998). In photosynthesis, the light energy brought to the plant cells is used by the chloroplasts to produce glucose ($C_6H_{12}O_6$) and oxygen (O_2) from carbon dioxide (CO_2). Aerobic respiration requires O_2 in order to produce ATP from $C_6H_{12}O_6$ as follow:

- $6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$
- $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ATP$

Plant cells produce reactive oxygen species (ROS) in chloroplasts and mitochondria as by-products of photosynthesis and respiration (Pitzschke et al. 2006). Under field stress conditions, plants also produced ROS in response to more than one environmental factor at a time. Overproduction of ROS leads to a degradation of key photosynthetic regulatory proteins and can trigger programmed cell death (Demmig-Adams and Adams 2002). Therefore, plants do not survive under extreme environmental stress without producing their own antioxidant defence systems (Foyer and Shigeoka 2011). Plants respond to ROS-generating abiotic (UV radiation, temperature, drought, soil moisture and salinity) and biotic (herbivore, pathogens) environmental stress factors by increasing the synthesis of a wide variety of phytochemicals, also known as secondary metabolites (Suzuki et al. 2014). Phytochemicals are low molecular weight non-enzymatic antioxidants including ascorbate (vitamin C), tocopherols (vitamin E), beta-carotenoids, glutathione and polyphenolic compounds (Bartley and Scolnik 1995; Decker 1997; Noctor and Foyer 1998; Wheeler et al. 1998; Demmig-Adams and Adams 2002; Munné-Bosch and Alegre 2002) that play important roles in the protection of plants against environmental threats and oxidative stress (Ahmad et al. 2010; Gill and Tuteja 2010; Choudhury et al. 2013; Kasote et al. 2015). Plants, plant-based whole foods and plant-derived products contain multitude phytonutrient antioxidants (Asensi-Fabado and Munné-Bosch 2010; Bolling et al. 2010; Chon 2013; Benzie and Choi 2014; Kasote et al. 2015; Škrovánková et al. 2015; Pisoschi et al. 2016; Van Hung 2016). Climatic and seasonal variations, regions of growth, degree of maturity, agriculture practices and postharvest treatment and processing influence the quality of plants and their antioxidant contents (Bolling et al. 2011; Škrovánková et al. 2012; Hur et al. 2014; Wang et al. 2014; McSweeney and Seetharaman 2015; Villa-Rodriguez et al. 2015; Kamiloglu et al. 2016).

1.3 Antioxidants and Phytotherapy

The healthcare systems in developed, developing, underdeveloping low-income countries have primarily focused on discovering and/or using medical drugs to treat and cure diseases. The production of drugs is a time-consuming process and extremely costly. Plants are easily accessible and a low-cost source of therapeutic molecules to a vast majority of world populations. Traditional medicine is now applied in different regions of the world depending on plant resources, human interaction with the natural environment, intercultural exchange and diffusion of traditional knowledge and population's socioeconomic status.

During the last decade, several studies emphasize the central role for ROS, oxidative stress and inflammation in the initiation and promotion of NCDs, including reproductive, gastrointestinal, hepatic, renal, pulmonary, cardiovascular, certain cancer and neurological diseases (Cachofeiro et al. 2008; Reuter et al. 2010; Perše 2013; Piechota-Polanczyk and Fichna 2014; Hawa et al. 2015; Tucker et al. 2015;

Verdile et al. 2015; Wiegman et al. 2015; Xu et al. 2015; Li et al. 2016). Phytochemicals have a wide range of biological activities such as antioxidative, anti-proliferative, anti-inflammatory and antiobesogenic and the concept of using plant antioxidants as effective agents to lower disease risk stemming from excessive production of ROS in the body (Upadhyay and Dixit 2015). Different antioxidants present in plant parts used in phytotherapy include roots, bark, leaves, flowers, fruit, seeds, berries as well as the whole plants, function synergistically and in combination with each other and are therefore much more effective in disease prevention and treatment.

The use of phytochemicals for health promotion, primary healthcare and disease prevention and treatment has gained increased interest worldwide particularly because of their availability, low cost and minimal side effects (WHO 2008; Cordell 2011; Rodriguez-Casado 2016; Shakya 2016). Whole plants are used by various ethnic cultures for their health benefits, as well as sources of medical drugs to prevent and treat NCDs (Howes and Houghton 2003; Abubakar et al. 2007; Ky et al. 2009; Ibarra-Alvarado et al. 2010; Alachkar et al. 2011; Kasabri et al. 2011, 2017; Hajdu and Hohmann 2012; Sawadogo et al. 2012; Semenya et al. 2012; Assaf et al. 2013; Al-Asmari et al. 2014; Kadir et al. 2014; Rokaya et al. 2014; Shil et al. 2014; Subramoniam 2014; Chege et al. 2015; Goyal 2015; Shweta and Boaz 2015; Stanifer et al. 2015; Apaya et al. 2016; Giovannini et al. 2016; Hitziger et al. 2016; Jacobo-Herrera et al. 2016; Ju et al. 2016; Kaufmann et al. 2016; Rastogi et al. 2016; Suroowan and Mahomoodally 2016; Zarshenas et al. 2016a, b; Tandon and Yadav 2017).

1.4 Healthy Plant-Based Diet

A healthy diet must promote health and reduce the incidence and development of nutrition-related disorders and chronic diseases. A healthy plant-based diet comprises essentially of a variety of fruit and vegetables, including legumes, beans, seeds, whole grains and nuts, on one hand, and limited animal products, added saturated and trans fats and refined carbohydrates, on the other hand. This type of diet is believed to lower the risk of coronary artery disease in the Mediterranean and Asian countries (Willett 1994). Finally, a healthy plant-based diet must contain fresh and minimally processed fruit and vegetables, mainly those without pesticides and genetically modified organisms. Following World War II, the use of a wide range of pesticides, including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides and plant growth regulators aiming at protecting crops from pests in conventional agricultural systems and enhancing crop yields, has increased dramatically worldwide. The presence of a non-negligible amount of chemicals especially pesticide residues in fruit, vegetables and plant-derived products from the conventional agricultural systems is of a major health concern. Consumers are increasingly aware of health risk associated with the consumption of pesticide-contaminated fruit, vegetables and plant-based foods.

Conventional agriculture cannot meet the nutritional needs of the current and ever-increasing human population without compromising the integrity of the environment. Pesticide residues are now found in soil, air and ground water. Environmental contamination by pesticide residues can adversely affect the life of many beneficial soil microorganisms, insects, plants, fish and birds (Aktar et al. 2009). Compared to conventional farming, organic agriculture is a sustainable production system that essentially maintains soil fertility by crop rotation, intercropping, polyculture, cover crops and mulching, and at the same time, it preserves biodiversity and environment and promotes human health. Although crop yields are 20% lower in the organic agricultural system than in the conventional agricultural system, input of fertilizer and energy was reduced by 34–53% and pesticide input by 97% (Mäder et al. 2002). A meta-analysis of studies provides evidence that organic agriculture enhances richness and abundance of plants, birds and predatory insects (Bengtsson et al. 2005). Over the past two decades, there has been a growing interest in organic products (Willer and Kilcher 2011). According to the International Federation of Organic Agriculture Movements (IFOAM), organic agriculture should be guided by four principles (Gomiero et al. 2011). The four principles of organic agriculture, established by the IFOAM (Luttikholt 2007), are:

- The Principle of Health—Organic agriculture should sustain and enhance the health of soil, plant, animal and human as one and indivisible.
- The Principle of Ecology—Organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them.
- The Principle of Fairness—Organic agriculture should build on relationships that ensure fairness with regard to the common environment and life opportunities.
- The Principle of Care—Organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment.

Besides providing healthy vegetables, the organic agriculture system is more sustainable than conventional agriculture system because they allow higher soil fertility and biodiversity (Gabriel et al. 2013), as well as being less dependent on external inputs. Many areas in different regions of the world such as Oceania, Europe and South and North America become organic farmland again.

1.5 Antioxidants and Prevention of Noncommunicable Chronic Diseases

Of the 57 million global deaths in 2008, 36 million were due to NCDs, and nearly 80% of NCD deaths occurred in low- and middle-income countries, of which 29% were among people under the age of 60 years (WHO 2011). The key facts regarding NCDs reported by WHO (WHO 2014) are:

- NCDs currently cause more deaths than all other causes combined, and NCD deaths are projected to increase from 38 million in 2012 to 52 million by 2030.
- Four major NCDs (cardiovascular diseases, cancer, chronic respiratory diseases and diabetes) are responsible for 82% of NCD deaths.
- Approximately 42% of all NCD deaths globally occurred before the age of 70 years; 48% of NCD deaths in low- and middle-income countries and 28% in high-income countries were in individuals aged under 70 years.

Unhealthy diets, alcohol consumption, tobacco smoking, second-hand smoke exposure and insufficient physical activity are the major risk factors for health disorders and NCDs, including obesity, hyperglycaemia, diabetes, hyperlipidaemia, raised blood pressure, cardiovascular diseases, respiratory diseases and cancers. Unhealthy nutritional patterns collectively termed the "Western dietary pattern", including intake of high-fat and high-cholesterol diets, fatty domestic red meats, refined vegetable oils, highly refined sugars and salt and consumption of processed, fast foods and alcohol, promote many of the chronic disorders and diseases, such as obesity, cardiovascular disease and cancer (Cordain et al. 2005). Contrary to the Western diet, the Mediterranean diet is low in saturated and trans fatty acids but contains plentiful plant foods, including fruit, vegetables, breads, nuts, seeds and olive oil. There is nowadays an increasing tendency to recommend regular intake of plant-based diets to forestall medical conditions, such as obesity, prenatal and postnatal disorders and complications, diabetes, cardiovascular disease, cancer, Alzheimer's disease (AD) and ageing (Tuso et al. 2013). Nutritional strategies for prevention of health disorders and NCDs are summarized below.

1.5.1 Early Life Origin of Obesity

The programming of adult obesity by intrauterine food restriction was identified 40 years ago in a historical cohort study of 300,000 men born from mothers exposed to the Dutch famine of 1944–1945 (Ravelli et al. 1976). Currently, obesity is a global public health threat that affects people of all ages, sexes and racial/ethnic groups (Chan and Woo 2010) that contributes to a global burden of NCDs and mortality (Savini et al. 2013). Widespread overweight and obesity were estimated to affect nearly 1.5 billion adults in 2008 (Popkin et al. 2012) and more than 1.9 billion adults in 2014 (WHO 2015). The key facts regarding obesity and overweight reported by WHO (WHO 2015) are:

- Worldwide obesity has more than doubled since 1980.
- In 2014, more than 1.9 billion adults, 18 years and older, were overweight. Of these over 600 million were obese.
- 39% of adults aged 18 years and over were overweight in 2014, and 13% were obese.

- Most of the world's population live in countries where overweight and obesity kill more people than underweight.
- Forty-one million children under the age of 5 were overweight or obese in 2014.
- Obesity is preventable.

Obesity is characterized by mitochondrial dysfunction, low antioxidant defences, increased ROS production, systemic oxidative stress, organ dysfunction and health disorders (Fernández-Sánchez et al. 2011), including hyperlipidaemias, atherosclerosis, hypertension, insulin resistance, hyperglycaemia and inflammation, which in combination play a significant role in the development and progression of NCDs, such as infertility, type 2 diabetes, non-alcoholic fatty liver disease, obstructive sleep apnea, coronary artery disease, stroke, peripheral arterial disease, cardiomyopathy, congestive heart failure and cancer (Savini et al. 2013). Maternal obesity also increases the risk of a number of pregnancy complications, including gestational diabetes, gestational hypertension, foetal macrosomia, large for gestational age, postpartum retention and perinatal morbidity and mortality (Moussa et al. 2016). Maternal obesity and excessive gestational weight gain influence foetal development and contribute to long-term metabolic consequences of children born to obese pregnant mothers (Stang and Huffman 2016). Faced by the rising prevalence of obesity and its related prenatal and postnatal disorders and complications that potentially predispose the infant to adult disease through foetal programming (Moussa et al. 2016), there is an urgent need for lifestyle and nutritional strategies to promote general health and prevent obesity, including weight loss, physical activity and a shift towards plant-based antioxidant-rich diets.

Periconceptional nutrition and dietary antioxidants are important for early developmental processes, mainly embryogenesis, placentation and foetal health and organ development (Cetin et al., 2010; Mistry and Williams 2011; Twigt et al. 2012; Al-Gubory 2013). Nutritional strategies before and during pregnancy that combat environmental factor-induced maternal and foetal oxidative stress can promote prenatal development, improve foetal health and reduce the onset and development of pathologies in adulthood. An interesting strategy is the adherence to the Mediterranean-type dietary pattern that enhances fertility (Toledo et al. 2011). Therefore specific nutritional formulas that are designed to reduce oxidative stress need to be developed and promoted for obese patients. Further research is needed to establish whether intake of a plant-based antioxidant-rich diet can prevent obesity before and during pregnancy and its associated prenatal and postnatal health complications and disorders.

1.5.2 Diabetes

Over the last three decades, the epidemic of diabetes has become a major worldwide public health concern. Diabesity, an association of obesity and diabetes, is the leading cause of NCDs. Type 2 diabetes and associated conditions known as "metabolic

syndrome" increased dramatically worldwide particularly due to changes in lifestyle behaviours, including a significant decline in physical activity and frequent sedentary behaviour (Zimmet et al. 2001). Obesity is associated with an increased risk of developing insulin resistance, dysfunction of pancreatic islet beta cells and failure to control blood glucose levels and type 2 diabetes (Kahn et al. 2006). The key facts regarding diabetes reported by WHO (WHO 2016a) are:

- The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014.
- The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014.
- Diabetes prevalence has been rising more rapidly in middle- and low-income countries.
- Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation.
- In 2012, an estimated 1.5 million deaths were directly caused by diabetes, and another 2.2 million deaths were attributable to high blood glucose.
- Almost half of all deaths attributable to high blood glucose occur before the age of 70 years. WHO projects that diabetes will be the seventh leading cause of death in 2030.
- Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use are ways to prevent or delay the onset of type 2 diabetes.
- Diabetes can be treated and its consequences avoided or delayed with diet, physical activity, medication, regular screening and treatment for complications.

The cost of diabetes in the United States of America (USA) is estimated to be more than US$ 174 billion per year in 2007, and the US diabetes prevalence rate is likely to increase dramatically over the next 30 years (Boyle et al. 2010). The world cost of diabetes is more than US$ 827 billion (WHO 2016a). In addition to high cost of treating diabetes, synthetic antihyperglycaemic agents have adverse side effects, such as hypoglycaemia, weight gain and hepato-renal toxicity. Therefore, many efforts are ongoing to discover low-cost, plant-derived natural products with hypoglycaemic property. The antihyperglycaemic property together with antioxidant activity of fruit, fruit peels, leaves and bark of many plants selected on the basis of their availability is established in experimental animals (Nain et al. 2012; Shivanna et al. 2013; Colomeu et al. 2014; Ali et al. 2015; Gondi et al. 2015; Joshi et al. 2016; Keshari et al. 2016). Clinical trials and animal studies suggest that fruit flavonoids have a favourable effect in management of diabetes without compromising cellular homeostasis and importantly with minimal side effects on the body (Tanveer et al. 2017).

1.5.3 Cardiovascular Diseases

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels, including coronary heart disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic

heart disease, congenital heart disease and heart failure. The major causes of CVDs are unhealthy dietary habits and unhealthy lifestyle behaviours, such as tobacco smoking, alcohol consumption, insufficient physical activity and/or physical inactivity. The key facts reported by World Health Organization (WHO 2016b) regarding CVDs are:

- CVDs are the number one cause of death globally: more people die annually from CVDs than from any other cause.
- An estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke.
- Over three quarters of CVD deaths take place in low- and middle-income countries.
- Out of the 16 million deaths under the age of 70 due to NCDs, 82% are in low- and middle-income countries, and 37% are caused by CVDs.
- Most cardiovascular diseases can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol using population-wide strategies.
- People with cardiovascular disease or who are at high cardiovascular risk (due to the presence of one or more risk factors such as hypertension, diabetes, hyperlipidaemia or already established disease) need early detection and management using counselling and medicines, as appropriate.

A well-balanced and healthy diet is of utmost importance for prevention of CVDs (Verlangieri et al. 1985; Law and Morris 1998; Kromhout 2001). Plants and plant-derived products are rich sources of polyphenols that promote cardiovascular health. Inflammation underlies the molecular basis of atherosclerosis. Flavanols have a range of cardiovascular-protective properties by modulating pro-inflammatory cytokine production, eicosanoids synthesis and platelet activation (Selmi et al. 2008). The anti-inflammatory and antioxidant effects of cocoa flavanols and other phenolic compounds explain, at least in part, the beneficial effects of cocoa on blood pressure, activity of platelets and leukocytes, flow-mediated vasodilatation and/or vascular function that collectively improve cardiovascular health and/or reduce CVD risk in healthy adults (Fisher et al. 2003; Engler et al. 2004; Heptinstall et al. 2006; Schroeter et al. 2006; Shiina et al. 2009), healthy smokers (Hermann et al. 2006), hypertensives (Grassi et al. 2005, 2008), hypercholesterolaemic postmenopausal women (Wang-Polagruto et al. 2006) and overweight men and women (Esser et al. 2014; West et al. 2014). A prospective study using data from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort suggests that higher chocolate intake is associated with a lower risk of future cardiovascular events among healthy men and women (Kwok et al. 2015). A Japanese cohort studies support a significant inverse association between chocolate consumption and risk of developing stroke in women (Dong et al. 2017).

Adoption of the Mediterranean diet reduces the risk of developing coronary heart disease in the elderly (Dontas et al. 2007). Adherence to a Mediterranean diet increases plasma levels of carotenoids, vitamin A and vitamin E (Azzini et al. 2011). The antioxidant (Mancini et al. 1995; Ghiselli et al. 1997) and anti-inflammatory

(Mena et al. 2009) effects of the Mediterranean dietary pattern can explain the low incidence of coronary heart in Mediterranean (Estruch et al. 2006, 2013; Turati et al. 2015) and non-Mediterranean populations (Tektonidis et al. 2015; Tong et al. 2016; Stefler et al. 2017).

1.5.4 Cancer

Globally, the number of people with cancer is projected to double by the year 2030, with most of this increase likely to occur in middle- and low-income countries of Africa and Asia (WCRF 2007). Almost 90–95% of cancers have their roots in exposure to environmental pollutants including heavy metals, particulate matter, ozone, sulphur oxides, carbon monoxide and nitrogen oxides (Sørensen et al. 2003; Huang et al. 2004; Møller et al. 2008; Wise et al. 2008; Yang and Omaye 2009) and also due to unhealthy lifestyle behaviours (Anand et al. 2008), including intake of diet high in fats, free sugars, salt, processed foods and red meat, as well as cigarette smoking, alcohol consumption and physical inactivity (Irigaray et al. 2007; Anand et al. 2008). Therefore, cancer is a preventable disease that requires major changes in lifestyle behaviours. The key facts regarding cancer reported by WHO (WHO 2017) are:

- Cancer is one of the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases in 2012.
- The number of new cases is expected to rise by about 70% over the next two decades.
- Cancer is the second leading cause of death globally and was responsible for 8.8 million deaths in 2015. Globally, nearly one in six deaths is due to cancer.
- Approximately 70% of deaths from cancer occur in low- and middle-income countries.
- Around one third of deaths from cancer are due to the five leading behavioural and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use and alcohol use.
- Tobacco use is the most important risk factor for cancer and is responsible for approximately 22% of cancer deaths.
- Cancer-causing infections, such as hepatitis and human papilloma virus (HPV), are responsible for up to 25% of cancer cases in low- and middle-income countries.
- Late-stage presentation and inaccessible diagnosis and treatment are common. In 2015, only 35% of low-income countries reported having pathology services generally available in the public sector. More than 90% of high-income countries reported treatment services are available compared to <30% of low-income countries.
- The economic impact of cancer is significant and is increasing. The total annual economic cost of cancer in 2010 was estimated at approximately US$ 1.16 trillion.

- Only one in five low- and middle-income countries have the necessary data to drive cancer policy.

The suggested cancer-protective effect of fruit and vegetable antioxidants is attributed to their ability to scavenge ROS and prevent DNA damage and subsequent mutation (Collins 1999). A balanced plant-based diet with a variety of fruit and vegetables can lower the risk for many types of cancer (La Vecchia et al. 2001; Donaldson 2004; Vainio and Weiderpass 2006; Liu et al. 2013; Maasland et al. 2015; Wang et al. 2015; Mut-Salud et al. 2016; Vieira et al. 2016). Polyphenols and carotenoids are of particular interest because they can reduce the risk of prostate (Giovannucci et al. 1995; Davalli et al. 2012), lung (Mayne et al. 1994; Shareck et al. 2017), breast (Freudenheim et al. 1996; Eliassen et al. 2012; Braakhuis et al. 2016; Yan et al. 2016), colon (Slattery et al. 2000), ovary (Zhang et al. 2007), liver (Darvesh et al. 2012), cervical (Di Domenico et al. 2012), oesophagus (Ge et al. 2013) and pancreas (Huang et al. 2016) cancer. Antioxidant compounds in plant extracts can play a preventative role against oxidative stress-induced mutation of DNA and development of cancer (Makhafola et al. 2016). In Europe, the Mediterranean diet is an important nutritional pattern of choice for cancer prevention (Giacosa et al. 2013; Grosso et al. 2013). The Mediterranean diet is naturally rich in chemopreventive agents, including polyphenols and carotenoids (Pelucchi et al. 2009; Scoditti et al. 2012), and may play a role in preventing skin (Fortes et al. 2008), breast (Trichopoulou et al. 2010; Hoffmann and Schwingshackl 2016), uterus (Filomeno et al. 2015), lung (Hodge et al. 2016) and colorectal (Rosato et al. 2016) cancer.

1.5.5 Ageing and Alzheimer's Disease

The free radical theory of ageing postulates that accumulated insults of biological systems during the ageing process induce damage of vital cellular macromolecules, which contribute to the decline in organ functional efficiency and onset of age-related complications and diseases (Harman 1956). Ageing is characterized by a decline in the efficiency of antioxidative defences and a progressive inability of organs to defend against environmental stressors. The accumulation of cellular ROS and the consequent oxidative modification of biological molecules have been proposed as responsible for the ageing process (Finkel and Holbrook 2000). Mitochondrial DNA (mtDNA) mutations and deletions play a role in the ageing process (Sastre et al. 2003; Lee and Wei 2012).

Life expectancy has markedly increased (~27 years) during the last three decades, mainly in developed and developing countries (Hayflick 2000). Therefore, health maintenance of the ageing population has become an economic and social concern. In addition, progressive loss of the ability to walk is associated with a decline in physical and social activities and a loss of independence. Longer lifespan will lead to a considerable increase in medical spending. Alzheimer's disease (AD) is the most common neurodegenerative disease in the

elderly. The lack of an effective treatment of AD is a real challenge due to its rising prevalence, and therefore preventive strategies of this chronic disease become a priority in many countries. AD patients have lower plasma levels of phytochemical antioxidants including folate vitamin A, vitamin B12, vitamin C and vitamin E (Jeandel et al. 1989; Lopes da Silva et al. 2014). Therefore, multiple antioxidant intervention strategies might be beneficial for the maintenance of a good cognitive function. In addition to the preventive effects of physical exercise against cognitive decline and the risk of dementia with age advancement (Paillard 2015), there is evidence suggesting that adopting healthy lifestyle behaviours and early intervention strategies, including intake of a balanced plant-based diet rich in antioxidants, can promote healthy ageing and may reduce risk for chronic age-associated diseases, mainly AD (Polidori et al. 2009; Arab and Sabbagh 2010; Shah 2013; Vassallo and Scerri 2013; Barnard et al. 2014).

1.6 Conclusions

Prevention of diseases requires a change in mindset from "live to eat" to "eat to live in a good health". This could prevent us from suffering the heavy physical and psychological consequences of many preventable and curable diet-related and/or lifestyle-related NCDs for which conventional medicinal and surgical care costs will be more and more expensive. Multiple strategies are needed to ensure and improve a healthy life, such as changes in dietary habits, food production and consumption and lifestyle behaviours. Physicians and many people are unaware of the potential benefits of healthy nutrition based on plants rich in antioxidant molecules. This is a challenging area of interest that holds important promise for the near future. Currently, the use of natural antioxidants in the prevention of oxidative stress-related NCDs is in its infancy and requires intensive investigations.

National and international policies should be directed towards sustainable agriculture to meet the need for healthy foods and diets. Feeding a growing world population; fighting hunger, undernourishment and malnutrition; preserving the environment and biodiversity; and producing healthy foods that promote health and prevent diseases are the major global challenges we face today. Another palpable challenge is climate change, which will reduce the availability of viable agricultural land. Under these conditions, the demand for water, agricultural land and food will increase considerably in the world. Last but not least, another major challenge for food security is the rapid growth of the world's population.

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Chapter 2
Plants of Indian Traditional Medicine with Antioxidant Activity

Abhishek Das, Dipankar Chaudhuri, Rhitajit Sarkar, Nikhil Baban Ghate, Sourav Panja, and Nripendranath Mandal

Abstract Oxidative stress is associated with increased production of reactive oxygen species (ROS) that can pose a threat to cells by causing lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition and activation of cell death pathways. An uncontrollable production of ROS may lead to organ dysfunction and diseases. It has been well documented in the last few decades that antioxidant compounds are the major agents that eliminate/scavenge ROS hence inhibiting oxidative stress and hindering the onset and development of non communicable chronic diseases (NCDs). Naturally occurring antioxidant compounds in plants may contribute to their potential dietary, nutritious and curative activities against ROS-induced oxidative cellular damage and NCDs. India is endowed with a variety of natural resources and flora with antioxidant principles that can be used in traditional medicine aimed at maintaining health and curing NCDs. Indian plants are important sources of alkaloids and phenolic compounds with potential antioxidant activities. Ancient texts of *Ayurveda* and *Charaka Samhita* mention innumerable herbal formulations in the treatment of NCDs that we know are caused due to oxidative stress and free-radical damage. Scientists around the world have shown interest in the Indian system of medicine and have realized the potential of Indian plants against ROS-induced cellular damage and NCDs. Plants mentioned in the texts of Indian traditional medicine are discussed here so as to project a picture of Indian flora as potential sources of antioxidants in the prevention and management of human NCDs.

Keywords Indian plants • Medical plants • Natural antioxidants • Trees • Shrubs • Herbs

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2.1 Introduction

Free radicals are produced either as a result of cell metabolism or after exposure of biological systems to environmental factors. Many diseases of ancient and contemporary times are believed to have been mediated by free radical-induced damage to cells (Pham-Huy et al. 2008). Our body has developed several antioxidant defence systems to limit damage from reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative stress results when generation of ROS supersedes cellular antioxidant defences. The detrimental results include the initiation of several diseased conditions in a human body, which tend to worsen with continuous exposure in due course of time. The development and wide application of chemical antioxidants (Li et al. 2007) have been constrained due to negative side effects and escalating costs. A wide array of secondary metabolites of herbal origin such as the phenolic compounds (phenolic acids, flavonoids, coumarins, quinines and other polyphenols), nitrogen compounds (alkaloids and amines), vitamins, terpenoids and other secondary metabolites have antioxidant activities (Gul et al. 2011). Naturally occurring antioxidant compounds are gaining prominence, and their identification in plants has promoted their potential dietary, nutritious and curative applications (Brewer 2011) against ROS-induced oxidative cellular damage and non communicable chronic diseases (NCDs) (Fig. 2.1).

India is endowed with an important variety of natural resources and flora with potential antioxidant activities useful in traditional medicine to maintain health and cure diseases (Scartezzini and Speroni 2000; Katiyar et al. 2013). Ancient *Ayurveda* "science of life" and its documented practices, an integral part of Indian culture and materia medica, indicated a pivotal role of several plants (Sivarajan and Balachandra 1996) in the treatment of various health concerns (Svoboda 1998; Dev 1999; Subhose et al. 2005; Rathore et al. 2007; Ven Murthy et al. 2010; Pandey et al. 2013) including some contagious diseases (Singh and Singh 2008). Various Indian plants have been also used in treatment of male reproductive disorders and diseases such as infertility, contraception, libido, sexually transmitted infections and reproductive tract cancers (Lohiya et al. 2016). The *Ayurveda* dates back to the period of the Indus Valley Civilization, about 3000 BC. (Ven Murthy et al. 2010). Now traditional herbal medicines represent more than 60 billion US$ in the global market. Their widespread availability, ease in procurement, low cost of processing and reduced risk of side effects are the hallmarks of their success as complementary or alternative therapies against NCDs.

There is evidence from rodents studies that herbal preparations such as Brahmarasayana, Narasimharasayana, Ashwagandharasayana, Amrithaprasham, Mentat and Abana have radioprotective effects and reduced radiation-induced ROS production and cellular damage in organs and tissues of biological systems (Saini et al. 1984; Kumar et al. 1996; Jagetia et al. 2002; Jagetia and Baliga 2003). In the *Charaka Samhita* (by *Charka* in 1500 BC), *Triphala*, an Ayurvedic formulation comprising *Terminalia chebula*, *Emblica officinalis* and *Terminalia bellirica*, is described as a *tridoshic rasayan* that has balancing and revivifying effects on *vata*,

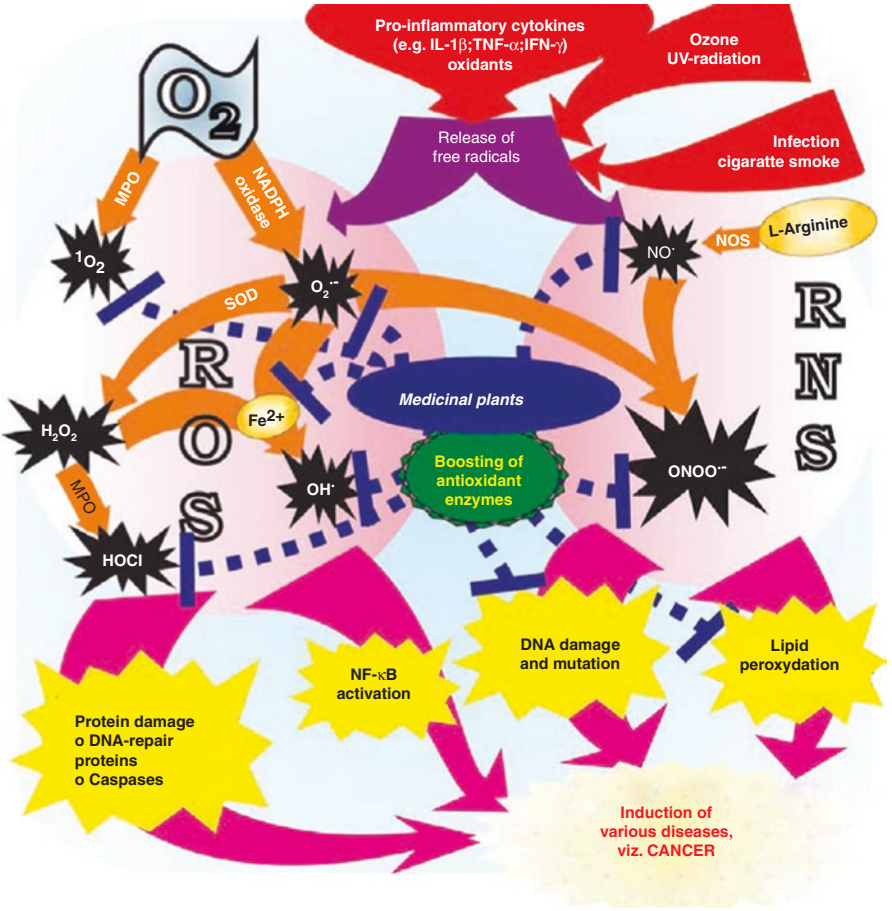


Fig. 2.1 Role of medicinal plants in prevention of diseases. Medicinal plants inhibit various diseases including iron overload, liver toxicity and cancer by reducing oxidative stress. Generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is initiated by respiratory bursts, which is initiated by various physiological and environmental factors. An assortment of ROS and RNS from molecular oxygen and L-arginine, respectively, formed by myeloperoxidase (MPO), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, superoxide dismutase (SOD) and nitric oxide synthase (NOS) leads to lipid peroxidation, DNA damage and then followed by mutation and nuclear factor-kappa B (NF-κB) activation. These phenomena give rise to wide range of diseases. Plant extracts exert their effect by inhibiting the formation and also scavenging the free radicals and non-radical ROS. Plant products also chelate iron and thus reduce iron overload-related pathological sequences

pitta and *kapha*, the three elements that constitute human life (Sharma and Dash 1998). The plants of *Triphala* have been proven useful source of natural antioxidants and their possible use in mitigating NCDs (Hazra et al. 2010b).

2.2 Reactive Oxygen Species

ROS are implicated in receptor-mediated signalling pathways (Knebel et al. 1996) as well as in transcriptional activation (Schreck et al. 1991). Oxidative stress is associated with increased production of ROS that can pose a threat to cells by causing lipid peroxidation, protein oxidation, nucleic acids damage, enzyme inhibition and activation of cell death pathways. Potentially harmful ROS include hydroxyl radical ($HO^{\bullet}$), superoxide radical ($^{\bullet}O_2^-$), peroxyl radical ($RO_2^{\bullet}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$). $HO^{\bullet}$ is produced from the decomposition of hydroperoxides (ROOH), radiation of atomic O_2, UV-light dissociation of H_2O_2 and likely during Fenton chemistry where trace amounts of reduced transition metals catalyse peroxide-mediated oxidations of organic compounds. $HO^{\bullet}$ has a very short in vivo half-life of a few seconds, even though it is extremely reactive (Sies 1993) by causing damage to virtually all types of macromolecules, including carbohydrates, nucleic acids, lipids and amino acids. High levels of H_2O_2 can attack several cellular energy-producing systems through $HO^{\bullet}$ in the presence of transition metal ions. Even though not a radical, HOCl is considered a potent chlorinating and oxidizing agent. The formation of cholesterol chlorohydrins could further disrupt cell membranes and lead to cell lysis and death (Carr et al. 1996). HOCl attacks primary amines and sulfhydryl groups in proteins and chlorinates purine bases in DNA (Dennis et al. 1979). Similarly, $^1O_2$ is not a true radical either but is believed to be an important ROS in reactions involving UV exposure. Its toxicity is increased by photosensitization with molecular oxygen. The presence of metals such as iron increases the production of $^1O_2$, as well as $^{\bullet}O_2^-$, thus accelerating the oxidation of unsaturated lipids. $^1O_2$ thus induces hyperoxidation and oxygen toxicity within cells (Kochevar and Redmond 2000).

2.3 Reactive Nitrogen Species

Common RNS include nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$). Nitric oxide is produced by a number of cell types, and sustained levels of production of this radical contribute to the vascular collapse associated with septic shock, whereas chronic production of $NO^{\bullet}$ is associated with various carcinomas and inflammatory conditions including diabetes, multiple sclerosis, arthritis and ulcerative colitis (Tylor et al. 1997). The toxicity of $NO^{\bullet}$ increases greatly when it reacts with $^{\bullet}O_2^-$, forming highly reactive $ONOO^-$ (Huie and Padmaja 1993). The relatively stable $ONOO^-$ and its protonated form, peroxynitrous acid (ONOOH), are highly reactive, cross biological membranes and undergo significant interactions with most cellular biomolecules (Pryor and Squadrito 1995). $ONOO^-$ can damage DNA by introducing oxidative modifications in both nucleobases and the sugar-phosphate backbone (Butler et al. 1998) and can also alter protein structure and function by reacting with

various amino acids in the peptide chain. The free radical $ONOO^-$ reacts with iron-sulphur clusters and inactivates enzymes implicated in critical metabolic processes (Castro et al. 1994) and triggering lipid peroxidation in cell membranes, liposomes and lipoproteins by removing a hydrogen atom from polyunsaturated fatty acids. These reactions contribute to the mechanisms of $ONOO^-$ cytotoxicity (Radi et al. 1991).

2.4 Medicinal Plants for Prevention of Chronic Diseases

Oxidative stress is involved in the pathogenesis of NCDs such as cancer, heart disease, diabetes mellitus, cataract formation and several neurodegenerative disorders (Qian et al. 2008). The burden of chronic diseases, like coronary heart disease (CHD), cancers, diabetes and obesity was found to contribute in 59% of the 56.5 million deaths worldwide in 2001, according to a World Health Organization (WHO) report (Mahady 2009). CHD comprises diseases of the circulatory system especially acute myocardial infarction, ischemic heart disease, valvular heart disease, peripheral vascular disease, arrhythmias and stroke (Mahady 2009). Medicinal sources from artichoke (*Cynara scolymus*), ginkgo (*Ginkgo biloba*), hawthorn (*Crataegus* spp.), garlic (*Allium sativum*), guggul (*Commiphora mukul*), red wine (*Vitis vinifera*), tea (*Camellia sinensis*) and saffron (*Crocus sativus*) are found to be promising dietary supplements in the prevention and treatment of CHD (Mahady 2009). Free radicals generated in our body have the tendency to manipulate a plethora of biomolecules causing these chronic health conditions. Functional plant foods/neutraceuticals are sources of antioxidants that can be consumed as beneficial diets in reducing the risk of chronic disorders such as obesity. It has been shown that *Nelumbo nucifera* extracts counteract obesity by inhibiting pancreatic lipase (Velusami et al. 2013) and also fastens healing in piles (Kalita et al. 2005).

Indian traditional practitioners of folk medicine have been using medicinal plants for treating acute and chronic diseases, since ages. Aerial parts of *Oxalis corniculata* Linn. and whole plant extract of *Leucas aspera* Spreng. are used for the treatment of diabetes (Kalita et al. 2005), whereas epilepsy is managed with the leaf extracts of *Lawsonia inermis* Linn. supplemented by cow milk (Kalita et al. 2005). Plants like *Oroxylum indicum* Vent., *Prunella vulgaris* Linn., *Sapindus mukorossi* Gaertn., *Syzygium cumini* (L.) Skeels., *Albizia chinensis* (Osb.) Merr., *Perilla frutescens* (L.) Britton and *Lasia spinosa* (L.) Thw. are used as folk remedies against impotency, skin problems, epilepsy, diabetes, snake bites, body swelling and helminthic infections, respectively (Jamir et al. 2012). *Aegle marmelos* Correa ex Roxb. extracts have been used for the treatment of abscess, heart disease and fever.

2.5 Indian Traditional Medicinal Plants and Antioxidants

Since the dawn of civilization, Indian plants (trees, shrubs and herbs) have been used as traditional medicines to cure various ailments as documented in ancient scripts of *Ayurveda*. Many plants possess compounds having large amounts of antioxidants with free radical scavenging activities. The types and parts of medicinal plants used are shown in Fig. 2.2. Plant roots, stems, barks, leaves, flowers, fruits and seeds have the potential for the treatment of several organ disorders and complications. The following is a summary of the experimental evidence for the free radical scavenging activities of medicinal plants and their health benefits.

The aim of this chapter is to scientifically establish the underlying principles of traditional Indian medicinal system, the *Ayurveda*, which regularly employs the practice of ingesting plant materials alone or as a mixture of plant materials in a form of a pill or powder. Sometimes, a solution (especially water and/or alcohol) of plant product or mixture of plant products is also used as a remedy in *Ayurveda*. It was evident that most of the plant-derived antioxidant compounds are either phenolics or flavonoids responsible for the bioactivity (Arabshahi-Delouee and Urooj 2007; Sultana et al. 2009; Sarkar et al. 2009a, b, 2014; Chaudhuri et al. 2016a, b).

Scientists are using different techniques to extract the active phenolics from the plant materials taking into account their chemistry and uneven distribution in the plant matrix. Among the different techniques, polarity-based solvent extraction is used most frequently. However, the antioxidant potential of a plant material mostly relies on the solvent used for extraction as the chemical properties and polarities vary for the different antioxidant compounds. So, it is important to check the extracts obtained from solvents of different polarities rather than solely depending on the most polar solvents only (Peschel et al. 2006). However, the results from different studies suggest that the aqueous mixtures containing ethanol, methanol, acetone and ethyl acetate extracts harbour most of the antioxidant compounds from the plants (Abdille et al. 2005; Rehman 2006; Li et al. 2006; Bonoli et al. 2004; Chatha et al. 2006; Siddhuraju and Becker 2003). These results eventually correlate the solvents used for extraction with their respective bioactivities as phenolics and flavonoids are

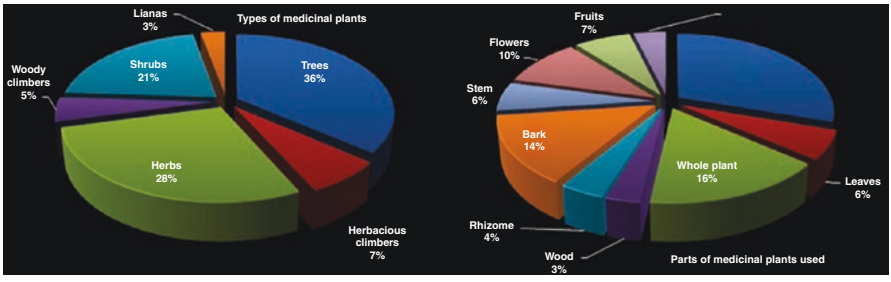


Fig. 2.2 Types and parts of Indian medicinal plants. About 70% of Indian medicinal plants grow in tropical and subtropical forests, and <30% are found in temperate and high-altitude forests. These medicinal plants belong to a wide range of plant types, including trees, herbs, lianas, woody climbers and twiners. In India, more than 90% of the plant species for industrial use are collected from the wild, and over 70% of this collection involves harvesting different parts of the plants

mostly extracted in ample amounts in high polar solvents such as aqueous methanol/ethanol as compared with absolute methanol/ethanol (Siddhuraju and Becker 2003; Anwar et al. 2006; Sultana et al. 2007). Therefore, the solvent(s) used plays an important role in determining the optimal medium of extraction of plant materials in a study of antioxidants and/or free radical scavenging properties.

2.5.1 Tree Antioxidant Activities

Two factors that differentiate trees from shrubs are (1) growing into a larger structure and (2) having a single well-defined main stem; however, the distinction between a small tree and a large shrub is not always clear (Lawrence and Hawthorne 2006). Trees are able to accumulate large quantities of carbon in their tissues by removing excess atmospheric carbon dioxide, reduce erosion, improve the climate, serve as a habitat for a diverse flora and fauna and provide food and timber and many other services to the biota. In addition, they are also a large reservoir of drugs as first described in early writings of traditional medicine. Parts of trees, including roots, barks, leaves, flowers, fruits and seeds, have been used by traditional practitioners and, recently, for identifying medicines against various diseases (Kumar et al. 2011a, b; Sharma et al. 2013). Findings of antioxidant and free radical activities from various tree parts are summarized in Table 2.1.

2.5.2 Shrub Antioxidant Activities

Shrubs are woody in nature (same as trees) but refrained in growth as they usually are under 6 m in height. Plants of several species can grow either into trees or shrubs, depending on their growing conditions including climatic and geographical restrictions. Shrubs in many parts of the world, including India, have antioxidant activities and are used in traditional medicine against many ailments (Argoti et al. 2013; Soysa et al. 2014; Anyanwu et al. 2015; Jarić et al. 2015). Much work has been done globally on shrubs used in Indian traditional medicine, some of which are mentioned in Table 2.2 according to the part of the plant used.

2.5.3 Herb Antioxidant Activities

Herbal plants are the shortest of the three forms of flora. Herbs are known for their beautiful foliage, aromatic traits, ornamental importance and numerous culinary purposes. Apart from being an important ingredient in human nutrition, herbs of Indian origin have therapeutic properties attributed to its natural phytochemical compounds and remain an integral part of the Indian traditional medicine for various biomedical applications (Sah et al. 2005; Gupta et al. 2011a, b, c; Mishra et al. 2011; Di Fabio et al. 2015; Ahmmed et al. 2016). Some of the findings related to their antioxidant activities are summarized in Table 2.3.

Table 2.1 Examples of antioxidant activities from different parts of trees

Name of the tree	Part of tree used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Prunus nepalensis Ser. (Steud)	Fruits	70% methanol	$ABTS^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $NO^{\bullet}$, $ONOO^-$, HOCL, iron chelation, SOD, CAT, GST, GSH, lipid peroxidation	Chaudhuri et al. (2015)
Ficus bengalensis L. (Indian banyan tree)	Arial roots	Methanol, 70% acetone	DPPH, $ABTS^{\bullet+}$, $^{\bullet}OH$, anti-haemolytic, iron chelation, reducing power	Manian et al. (2008)
Erythrina indica	Roots	Methanol	Ferric reducing antioxidant power, DPPH, $NO^{\bullet}$, $^{\bullet}O_2^-$	Sre et al. (2012)
Pandanus odoratissimus L.	Roots	Methanol, water	DPPH, reducing power	Sasikumar et al. (2009)
Azadirachta indica A. Juss Var., Meliaceae	Root bark	80% Ethanol	DPPH, Total antioxidant	Kiranmai et al. (2011)
	Flower	Water, methanol, ethanol	DPPH	Nahak and Sahu (2011)
	Seed oil	Nil		
Vitex trifoliate	Roots	Chloroform, methanol	$^{\bullet}O_2^-$, DPPH, lipid peroxidation	Sreedhar et al. (2010)
Aporosa lindleyana Baill	Roots	Petroleum ether (40–60 °C), chloroform, ethyl acetate, methanol, 50% methanol, water	DPPH, $NO^{\bullet}$, CAT, SOD, lipid peroxidation	Badami et al. (2005)
Mesua ferrea Linn.	Roots	*n*-Hexane, dichloromethane, ethyl acetate, methanol	DPPH	Teh et al. (2013)
	Stem bark	Chloroform, ethanol	Total antioxidant, DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, anti-haemolytic, DNA protection	Rajesh et al. (2013)
	Flowers	Petroleum ether (60–80 °C), chloroform, methanol	Total antioxidant, DPPH, $^{\bullet}O_2^-$, H_2O_2	Sahu et al. (2013)
	Seed oil	Petroleum ether	DPPH, $ABTS^{\bullet+}$, $NO^{\bullet}$	Chahar et al. (2012)

Bombax ceiba Linn	Roots	Methanol	DPPH radicals, total antioxidant status, reducing power	Jain et al. (2011)
	Stem bark	95% Ethanol, water	Total antioxidant, DPPH, $^{\bullet}O_2^-$, NO$^{\bullet}$, $ABTS^{\bullet+}$, SOD, lipid peroxidation, reducing power	Gandhare et al. (2010)
	Flowers	50% ethanol, 80% acetone	DPPH, total antioxidant, reducing power	Yu et al. (2011)
Pistacia integerrima Stewart	Galls	Ethanol, *n*-hexane, chloroform, ethyl acetate, methanol	DPPH	Rauf et al. (2014)
	Leaves			
	Stem bark			
	Roots			
	Fruits	70% methanol	DPPH	Ilahi et al. (2013)
Bauhinia variegata	Stem bark	95% ethanol, water	$^{\bullet}O_2^-$, H_2O_2, DPPH, NO$^{\bullet}$, Reducing power	Rajani and Ashok (2009)
	Roots			
Acacia catechu (L.f.) wild	Heartwood	70% methanol	$ABTS^{\bullet+}$, DPPH, $^{\bullet}OH$,$^{\bullet}O_2^-$, NO$^{\bullet}$, $ONOO^-$, H_2O_2, HOCl, lipid peroxidation, DNA protection, iron chelation	Hazra et al. (2010a)
Spondias pinnata	Stem bark	70% methanol	$ABTS^{\bullet+}$,$^{\bullet}O_2^-$,$^1O_2$, NO$^{\bullet}$, $^{\bullet}OH$, $ONOO^-$, H_2O_2, HOCl, lipid peroxidation, iron chelation, reducing power	Hazra et al. (2008)
Ficus racemosa L.	Stem bark	Methanol, 70% acetone	DPPH, $ABTS^{\bullet+}$, $^{\bullet}OH$, anti-haemolytic, iron chelation, reducing power	Manian et al. (2008)
Caesalpinia sappan	Heartwood	Petroleum ether (40–60 °C), chloroform, ethyl acetate, methanol, 50% methanol, water	DPPH, levels of CAT, SOD, lipid peroxidation	Badami et al. (2003a)

(continued)

Table 2.1 (continued)

Name of the tree	Part of tree used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Cassia fistula L.	Flowers	90% methanol	$^{\bullet}O_2^-$, DPPH, liposome peroxidation, reducing power	Siddhuraju et al. (2002)
		Hydroalcohol	DPPH, reducing power	Bhalodia et al. (2011)
	Fruit pulp	90% methanol	$^{\bullet}O_2^-$, liposome peroxidation, reducing power	Siddhuraju et al. (2002)
		Hexane, methanol	DPPH, $^{\bullet}OH$, FRAP total antioxidant, reducing power	Irshad et al. (2012)
	Leaves	90% ethanol	$^{\bullet}O_2^-$, DPPH, liposome peroxidation, reducing power	Siddhuraju et al. (2002)
	Stem bark	Petroleum ether (60–80 °C), methanol	DPPH, $NO^{\bullet}$	Jagtap and Pal (2010)
		90% methanol	$^{\bullet}O_2^-$, DPPH, liposome peroxidation, reducing power	Siddhuraju et al. (2002)
	Seeds	Hexane, methanol	DPPH, $^{\bullet}OH$, FRAP total antioxidant, reducing power	Irshad et al. (2012)
Ficus microcarpa	Stem bark	Ethyl acetate	DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$	Ao et al. (2008)
	Leaves			
	Fruits			
Polyalthia longifolia Benth. and Hook	Stem bark	Ethanol	DPPH, lipid peroxidation, reducing power	Manjula et al. (2010)
Prunus cerasoides D. Don	Stem bark	80% ethanol	DPPH, FRAP total antioxidant	Guleria et al. (2013a)
Abies spectabilis (D. Don) Spach.	Stem bark	Methanol, chloroform	DPPH, $ABTS^{\bullet+}$, iron chelation	Dall'Acqua et al. (2012)
	Leaves	70% ethanol	DPPH, H_2O_2, $^{\bullet}OH$, $NO^{\bullet}$, reducing power	Tote et al. (2009)

Albizia lebbeck (L.) Benth	Stem bark	Methanol	DPPH, reducing power	Suruse et al. (2013)
	Pods	80% methanol	Anti-haemolytic, $ABTS^{\bullet+}$, FRAP and TRAP total antioxidant	Zia-ul-Haq et al. (2013)
	Seeds			
Aesculus hippocastanum L.	Stem bark	Ethanol	DPPH, lipid peroxidation	Celep et al. (2012)
	Leaves			
	Flowers			
	Seeds			
Anogeissus latifolia	Stem bark	50% ethanol	DPPH, $^{\bullet}O_2^-$, H_2O_2, $NO^{\bullet}$, lipid peroxidation	Govindarajan et al. (2004)
Crataeva nurvala Buch. Ham.	Stem bark	Chloroform, ethyl acetate, acetone, methanol	DPPH	Raut and Gaikwad (2014)
Shorea roxburghii	Stem bark	Acetone, methanol	DPPH, H_2O_2, $^{\bullet}OH$, $ABTS^{\bullet+}$, reducing power	Subramanian et al. (2013)
Shorea robusta Gaertn	Stem bark	Water	DPPH	Guerrero et al. (2004)
Aphanamixis polystachya	Stem bark	Ethanol	DPPH	
Semecarpus anacardium L.	Stem bark	Hexane, chloroform, ethyl acetate, methanol	DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, lipid peroxidation	Sahoo et al. (2008)
	Leaves	Acetone, chloroform, ethanol, water	$ABTS^{\bullet+}$, DPPH, iron chelation	Barman et al. (2013)
	Nuts			
	Seeds	Ethanol	DPPH	Guerrero et al. (2004)
Bauhinia purpurea L.	Leaves	Hexane, ethyl acetate, methanol	DPPH, $NO^{\bullet}$	Urmi et al. (2013)
	Stem bark			
Saraca asoca (Roxb.) De Wild	Stem bark	60% ethanol, 90% ethanol, acetone	DPPH	Panchawat and Sisodia (2010)
	Leaves	Petroleum ether (60–80 °C), chloroform, methanol	DPPH	Kumar et al. (2012)

(continued)

Table 2.1 (continued)

Name of the tree	Part of tree used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Saraca indica	Stem bark	Hexane, chloroform, ethyl acetate, ethanol, water	FRAP total antioxidant, DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, lipid peroxidation	Gayathri and Jeyanthi (2013)
	Leaves	Petroleum ether (60–80 °C), chloroform, methanol	DPPH, $^{\bullet}O_2^-$, H_2O_2, $^{\bullet}OH$, $NO^{\bullet}$, lipid peroxidation, anti-haemolytic	Sen et al. (2014)
Pongamia pinnata (L) Pierre	Leaves	70% methanol	$ABTS^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, $ONOO^-$, $^1O_2$, HOCl, lipid peroxidation, iron chelation, reducing power	Hazra et al. (2011)
	Flowers			
	Seeds			
Moringa oleifera Lam.	Leaves	70% ethanol, 80% methanol, water	DPPH, $^{\bullet}O_2^-$, lipid peroxidation, reducing power	Siddhuraju and Becker (2003)
	Leaves	80% ethanol	FRAP total antioxidant, DPPH, levels of SOD, CAT, GSH, MDA	Fakurazi et al. (2012)
	Stems			
	Pods			
	Flowers			
	Seeds			
Momordica dioica Roxb.	Leaves	95% ethanol, water	DPPH, levels of SOD, CAT, GSH, MDA	Jain et al. (2008)
Mangifera indica L.	Leaves	98% methanol	DPPH, FTC, TBA, total antioxidant	Aqil et al. (2006)
Lawsonia inermis L.				
Sesbania grandiflora L. Pers	Leaves	Tris HCl buffer (pH 7.0)	DPPH, $^{\bullet}OH$, lipid peroxidation, iron chelation, reducing power	Padmaja et al. (2011)
Aegle marmelos L. Correa ex Roxb.	Leaves	80% methanol	FRAP total antioxidant, DPPH	Guleria et al. (2013a)
Cinnamomum camphora L. T. Nees and C. H. Eberm.				
Taxus baccata L.				

Abies pindrow Royle	Leaves	Dichloromethane, methanol, acetone	$ABTS^{\bullet+}$, DPPH, FRAP total antioxidant, $^{\bullet}O_2^-$, iron chelation, reducing power	Gupta et al. (2011a)
Acacia arabica	Leaves	Methanol, 100% methanol, water	DPPH, H_2O_2	Aadil et al. (2012)
	Seeds	Acetone	DPPH, $^{\bullet}O_2^-$, $NO^{\bullet}$	Parmar et al. (2010)
Acacia pennata	Leaves	Methanol	DPPH	Nanasombat and Teckchuen (2009)
Ailanthus excels (Roxb.)	Leaves	70% methanol	DPPH, FRAP total antioxidant	Said et al. (2010)
Albizia procera	Leaves	Methanol, petroleum ether, dichloromethane, carbon tetrachloride, ethyl acetate, water	DPPH, phosphomolybdate assay, reducing power	Khatoon et al. (2013)
Anacardium occidentale	Leaves	Hexane, 95% ethanol, water	DPPH	Ifesan et al. (2013)
Carica papaya				
Commiphora caudate	Leaves	Ethanol	DPPH, $^{\bullet}O_2^-$, $NO^{\bullet}$, lipid peroxidation, reducing power	Deepa et al. (2009)
Commiphora var pubescens				
Zanthoxylum alatum Roxb.	Leaves	Essential oil, chloroform, ethyl acetate, acetone, methanol	DPPH, iron chelation, reducing power	Guleria et al. (2013b)
	Fruits	95% ethanol	DPPH, $^{\bullet}OH$, iron chelation, phosphomolybdenum reduction assay	Batool et al. (2010)
Butea monosperma Lam.	Leaves	Petroleum ether, chloroform	DPPH, $NO^{\bullet}$	Borkar et al. (2008)
	Flowers	Ethyl acetate, *n*-butanol, methanol, water	DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, anti-haemolytic	Lavhale and Mishra (2007)
Randia dumetorum	Leaves	70% ethanol	DPPH, $^{\bullet}O_2^-$, iron chelation, phosphomolybdenum reduction assay, reducing power	Gandhimathi and Bai (2013)
Delonix regia Gamble.	Flowers	98% methanol	DPPH, FTC, TBA, total antioxidant	Aqil et al. (2006)
Peltophorum ferrugineum	Flowers	Hexane, ethyl acetate, acetone, methanol	DPPH, phosphomolybdenum reduction assay, reducing power	Pavagadhi et al. (2012)

(continued)

Table 2.1 (continued)

Name of the tree	Part of tree used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Lagerstroemia speciosa	Flowers	Methanol	DPPH, reducing power	Pavithra et al. (2013)
Wendlandia thyrsoidea				
Bombax malabaricum				
Olea dioica				
Wrightia tinctoria (Roxb.)	Flowers	95% ethanol	DPPH, H_2O_2, iron chelation, phosphomolybdenum reduction assay, reducing power	Ramalakhsmi et al. (2012)
Punica granatum L.	Flowers	1% HCl in methanol	DPPH, $ABTS^{\bullet+}$	Zhang et al. (2011)
	Fruit rind	98% methanol	DPPH, FTC, TBA, total antioxidant	Aqil et al. (2006)
Populus nigra	Flowers buds	Ethanol, hexane, ethyl acetate, chloroform, water	DPPH, $ABTS^{\bullet+}$, H_2O_2, $^{\bullet}OH$, $NO^{\bullet}$, HOCl, lipid peroxidation	Debbache et al. (2014)
Michelia champaca	Flowers	Hexane, ethyl acetate	DPPH	Parimi and Kolli (2012)
Litchi chinensis	Flowers	Hexane, ethyl acetate, *n*-butanol, acetone	DPPH	Yang et al. (2012)
Nerium oleander	Flowers	Petroleum ether, chloroform, ethyl acetate, methanol, water	DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^{\bullet}OH$, iron chelation	Singhal and Gupta (2012)
Butea frondosa	Flowers	95% ethanol, methanol, water	DPPH, lipid peroxidation	Lal and Mantri (2011)
Castanea sativa	Leaves	Water	DPPH, β-carotene bleaching, anti-haemolytic, lipid peroxidation, reducing power	Barreira et al. (2008)
	Fruits			
	Flowers			

Terminalia chebula Retz.	Fruits	70% methanol	$ABTS^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, H_2O_2, $ONOO^-$, $^1O_2$, HOCl, lipid peroxidation; levels of SOD, CAT, GSH, GST, reducing power	Hazra et al. (2010b)
Terminalia belerica Roxb.				
Emblica officinalis Gaertn.				
Tamarindus indica	Coat	Petroleum ether, 70% acetone, methanol	$ABTS^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, FRAP total antioxidant	Siddhuraju (2007)
Areca catechu L.	Seed nuts	Petroleum ether, ethyl acetate, methanol, water, 50% methanol	H_2O_2, reducing power	Hannan et al. (2012)
Artocarpous heterolphyllus Lam	Seeds	50% dichloromethane in methanol, acetone	DPPH, $ABTS^{\bullet+}$, FRAP total antioxidant, iron chelation	Gupta et al. (2011b)
Hydnocarpus wightiana Blume.	Seed hulls	Petroleum ether, chloroform, acetone	DPPH, $ABTS^{\bullet+}$, α-glucosidase inhibitory	Reddy et al. (2005)
Alstonia scholaris Linn.	Leaves	Methanol	DPPH, $^{\bullet}O_2^-$, iron chelation, reducing power	Ganjewala and Gupta (2013)
	Follicles			
	Flowers	Hexane, benzene, methanol, water	DPPH, β-carotene bleaching	James et al. (2011)
	Fruits			
Zanthoxylum armatum DC	Stem bark	Ethanol	DPPH	Sati et al. (2011)

Table 2.2 Examples of antioxidant activities from different parts of shrubs

Name of the shrub	Shrub part used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Elaeagnus latifolia Linn.	Fruits	70% methanol	ABTS$^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$	Panja et al. (2014)
Withania somnifera L. Dunal	Roots	70% methanol	ABTS$^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $^1O_2$, NO$^{\bullet}$, $^{\bullet}OH$, $ONOO^-$, HOCl, lipid peroxidation, reducing power	Chaudhuri et al. (2012)
Plumbago zeylanica L	Roots	97% methanol	DPPH, FTC, TBA, total antioxidant	Zahin et al. (2009)
	Stem Bark	Methanol	DPPH, FRAP total antioxidant	Suman et al. (2013)
Caesalpinia digyna	Roots	Petroleum ether (60–80 °C), methanol, water	ABTS$^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, H_2O_2, $^{\bullet}OH$, NO$^{\bullet}$, lipid peroxidation, levels of SOD, CAT, MDA	Srinivasan et al. (2007)
Coccinia grandis	Roots	70% methanol	DPPH, H_2O_2, NO$^{\bullet}$, reducing power	Bhadauria et al. (2012)
Asparagus racemosus Willd.	Roots	70% methanol, ethyl acetate, *n*-butanol, methanol, water	DPPH, levels of SOD, CAT, GSH, MDA	Acharya et al. (2012)
Abutilon indicum Linn.	Roots	Petroleum ether (60–80 °C), ethanol	DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, NO$^{\bullet}$, reducing power	Adikay et al. (2013)
	Stems	50% methanol water	DPPH	Chakraborthy and Ghorpade (2010)
	Flowers	70% ethanol	$^{\bullet}O_2^-$, $^{\bullet}OH$, reducing power	Revansiddaya et al. (2011)
Pothos scandens L.	Leaves	Petroleum ether, benzene, chloroform, ethyl acetate, acetone, methanol, ethanol	ABTS$^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, H_2O_2, NO$^{\bullet}$, phosphomolybdenum assay, FRAP total antioxidant, iron chelation	Sajeesh et al. (2011)
	Stem			
	Roots			
Althaea officinalis L.	Roots	50% ethanol, 70% ethanol, 90% ethanol, water	ABTS$^{\bullet+}$, HOCl, lipid peroxidation	Benbassat et al. (2014)
	Flowers	Ethanol	$^{\bullet}O_2^-$	Elmastas et al. (2003)

Glycyrrhiza glabra	Roots	Ethanol, water	$ABTS^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $NO^{\bullet}$, $^{\bullet}OH$, iron chelation, reducing power	Visavadiya et al. (2009)
Plumbago indica	Roots	Acetone, methanol	DPPH, $^{\bullet}OH$, phosphomolybdenum assay, reducing power	Eldhose et al. (2013)
Dioscorea alata L.	Modified stem	70% methanol	$ABTS^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $^1O_2$, $NO^{\bullet}$, $ONOO^-$, $^{\bullet}OH$, HOCl, lipid peroxidation, iron chelation, reducing power	Das et al. (2012)
	Leaves	70% methanol, water		Das et al. (2014)
Tinospora cordifolia	Stems	70% methanol	$ABTS^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $^1O_2$, $NO^{\bullet}$, $ONOO^-$, $^{\bullet}OH$, HOCl, lipid peroxidation, iron chelation, DNA protection	Ghate et al. (2013)
Hemidesmus indicus R. Br.	Stems	97% methanol	DPPH, FTC, TBA, total antioxidant assay	Zahin et al. (2009)
	Roots	70% methanol	$ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^1O_2$, $NO^{\bullet}$, $ONOO^-$, $^{\bullet}OH$, HOCl, lipid peroxidation, iron chelation, reducing power	Mandal et al. (2009)
Dioscorea pentaphylla L. Ver.	Tubers	Petroleum ether, benzene, ethyl acetate, methanol, ethanol	DPPH, $ABTS^{\bullet+}$, $^{\bullet}OH$, reducing power	Paulpriya and Mohan (2013)
Fagonia schweinfurthii (Hadidi)	Whole plants	Ethanol	DPPH, $ABTS^{\bullet+}$, H_2O_2	Pareek et al. (2013)
Caesalpinia crista Linn.	Leaves	70% methanol	$ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^1O_2$, $NO^{\bullet}$, $ONOO^-$, $^{\bullet}OH$, HOCl, iron chelation, reducing power	Mandal et al. (2011)
Cajanus cajan (L.) Millsp.	Leaves	70% methanol	$ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^1O_2$, $NO^{\bullet}$, $^{\bullet}OH$, $ONOO^-$, HOCl, iron chelation, lipid peroxidation, reducing power	Sarkar et al. (2009a)

(continued)

Table 2.2 (continued)

Name of the shrub	Shrub part used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Clerodendrum colebrookianum Walp.	Leaves	70% methanol, water	$ABTS^{\bullet+}$, DPPH, $^{\bullet}O_2^-$, $^1O_2$, $NO^{\bullet}$, $ONOO^-$, $^{\bullet}OH$, HOCl, lipid peroxidation, reducing power	Das et al. (2013)
Rauwolfia serpentina L. Benth. ex. Kurz	Leaves	Petroleum ether (20–80 °C), 80% acetone	$ABTS^{\bullet+}$, DPPH	Harisaranraj et al. (2009)
Indigofera tinctoria L.	Leaves	Petroleum ether, benzene, chloroform, ethyl acetate	DPPH, $ABTS^{\bullet+}$, $NO^{\bullet}$, $^{\bullet}OH$, iron chelation	Anusuya and Manian (2013)
Adhatoda vasica Nees	Leaves	Tris HCl buffer (pH 7.0)	DPPH, $^{\bullet}OH$, lipid peroxidation, iron chelation, reducing power	Padmaja et al. (2011)
Abroma augusta Linn.	Leaves	Methanol	DPPH	Bhuiya et al. (2013)
Abrus precatorius	Leaves	Hexane, ethyl acetate, ethanol, water	DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, H_2O_2, phosphomolybdenum assay, FRAP total antioxidant assay, lipid peroxidation	Gul et al. (2013)
	Seeds	Ethanol	H_2O_2, $^{\bullet}OH$, reducing power	Pal et al. (2009)
Sambucus nigra L.	Leaves	80% ethanol	DPPH, β-carotene antioxidant assay	Dawidowicz et al. (2006)
	Flowers			
	Fruits			
Artemisia vulgaris Linn.	Leaves, essential oil	Steam distillation	DPPH, iron chelation, FTC, total antioxidant assay, reducing power	Bhatt et al. (2007)
	Leaves	Methanol	DPPH, $ABTS^{\bullet+}$, $^{\bullet}OH$, H_2O_2	Sharmila and Padma (2013)
Musa paradicicus L.	Flowers	80% methanol	DPPH, $ABTS^{\bullet+}$, $^{\bullet}OH$, TBA, total antioxidant assay, lipid peroxidation, reducing power	China et al. (2011)
Embelia ribes	Flowers	Ethanol, water	$NO^{\bullet}$, FTC, total antioxidant assay, reducing power	Basavaraj and Ashok (2012)

Hibiscus rosa-sinensis L.	Flowers	Ethanol, water	DPPH, FRAP total antioxidant assay	Mak et al. (2013)
Senna bicapsularis				
Cassia auriculata	Flowers	Ethanol, methanol	DPPH, $ABTS^{\bullet+}$	Kumaran and Karunakaran (2007)
Dolichos biflorus Linn.	Seeds	70% methanol	$ABTS^{\bullet+}$, $^{\bullet}OH$, $^{\bullet}O_2^-$, $NO^{\bullet}$, HOCl, lipid peroxidation, reducing power	Hazra et al. (2009)
Hygrophila schulli (Buch.-Ham.)	Seeds	Ethanol	DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $NO^{\bullet}$, FRAP total antioxidant assay, phosphomolybdenum assay, reducing power	Lobo et al. (2010)
Solanum muricatum Aiton	Fruits	Ethyl acetate	DPPH, $ABTS^{\bullet+}$, $^{\bullet}OH$, phosphomolybdenum assay, iron chelation, FRAP total antioxidant assay, reducing power	Sudha et al. (2011)
Berberis asiatica	Fruits	Methanol	DPPH, $ABTS^{\bullet+}$, iron chelation, phosphomolybdenum assay, FRAP total antioxidant assay, reducing power	Pal et al. (2013)
Pyracantha crenulata				
Papaver somniferum L.		95% ethanol	DPPH	Baroš et al. (2012)
Carum copticum L.	Fruits	Methanol	DPPH, phosphomolybdenum assay	Zahin et al. (2010)
Foeniculum vulgare	Seeds	Ethanol, water	$^{\bullet}O_2^-$, H_2O_2, iron chelation, reducing power	Oktay et al. (2003)

Table 2.3 Examples of antioxidant activities from different parts of herbs

Name of the herb	Herb part used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Cichorium intybus L.	Roots	98% methanol	DPPH, FTC, TBA, total antioxidant	Aqil et al. (2006)
Hypochaeris radicata L.	Leaves	Petroleum ether, chloroform, ethyl acetate, methanol, water	DPPH, $ABTS^{\bullet+}$, iron chelation, reducing power	Jamuna et al. (2012)
	Roots			
Curcuma longa L.	Rhizomes	80% methanol	DPPH, FRAP total antioxidant	Guleria et al. (2013a)
Acorus calamus L.	Roots			
Acalypha indica Linn.	Leaves	Methanol	DPPH	Shanmugapriya et al. (2011)
	Roots			
Aconitum heterophyllum Wall	Roots	Ethanol	DPPH, H_2O_2, $NO^{\bullet}$, $^{\bullet}OH$, phosphomolybdate assay	Prasad et al. (2012)
Actaea spicata	Roots	Ethyl acetate, methanol	DPPH	Madaan et al. (2011)
Achyranthes aspera L.	Roots	Hexane, dichloromethane, ethyl acetate, methanol	DPPH, phosphomolybdate assay, reducing power	Rama et al. (2013)
Alpinia galanga L.	Rhizomes	Ethanol, acetone	$^{\bullet}O_2^-$, $^{\bullet}OH$, DPPH, phosphomolybdate assay	Divakaran et al. (2013)
Anacyclus pyrethrum	Roots	Ethanol	DPPH, H_2O_2, $NO^{\bullet}$, $^{\bullet}OH$, lipid peroxidation, reducing power	Sujith et al. (2011)
Argyreia speciosa (Burm.f) Boj.	Roots	Ethyl acetate, ethanol	DPPH, $^{\bullet}OH$, lipid peroxidation	Habbu et al. (2010)
	Leaves	Petroleum ether (60–80 °C), chloroform, methanol	DPPH, H_2O_2,$^{\bullet}O_2^-$, phosphomolybdate assay	Sahu et al. (2013)

Clitoria ternatea L.	Leaves	Methanol	DPPH, FRAP total antioxidant, iron chelation, reducing power	Jadhav et al. (2013)
	Stems			
	Roots			
	Flowers	95% ethanol, water	DPPH	Kamkaen and Wilkinson (2009)
	Seeds	Methanol	$^{\bullet}O_2^-$,$^{\bullet}OH$, DPPH, lipid peroxidation, reducing power	Jacob and Latha (2013)
Cissampelos parieta	Roots	50% ethanol	DPPH, $^{\bullet}O_2^-$, H_2O_2, $^{\bullet}OH$, $NO^{\bullet}$, levels of CAT, SOD, GST, GSH, lipid peroxidation, reducing power	Amresh et al. (2007)
Coscinium fenestratum	Leaves	Methanol	DPPH, $ABTS^{\bullet+}$	Goveas and Abraham (2013)
	Stems			
	Stems	Methanol	DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $NO^{\bullet}$, iron chelation, lipid peroxidation	Shirwaikar et al. (2007)
	Roots	Ethanol, water	DPPH, $NO^{\bullet}$, FRAP total antioxidant, reducing power	Basavaraj and Ashok (2012)
Medicago sativa L.	Roots	Methanol	DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $NO^{\bullet}$, iron chelation, lipid peroxidation	Rana et al. (2010)
Desmodium gangeticum	Roots	Ethyl acetate	DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, lipid peroxidation	Kurian et al. (2010)
Sida cordifolia Linn.	Whole plant	90% ethanol, water	$ABTS^{\bullet+}$, lipid peroxidation	Auddy et al. (2003)
Evolvulus alsinoides Linn.				
Maranta arundinacea L.	Tuberous rhizomes	Petroleum ether, ethyl acetate, ethanol	DPPH, $ABTS^{\bullet+}$, H_2O_2, $NO^{\bullet}$, FRAP total antioxidant, reducing power	Nishaa et al. (2012)
Striga Orobanchioides Benth.	Whole plant	Ethanol	DPPH, $NO^{\bullet}$ levels of CAT, SOD, lipid peroxidation	Badami et al. (2003b)

(continued)

Table 2.3 (continued)

Name of the herb	Herb part used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Phyllanthus simplex	Whole plant	Petroleum ether (60–80 °C), ethanol	DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, phosphomolybdate assay	Chouhan and Singh (2011)
Coleous vettiveroides (Jacob)	Whole plant	Methanol	DPPH, NO$^{\bullet}$, phosphomolybdate assay	Gopalakrishnan et al. (2011)
Coccinia indica	Stem bark	Methanol	DPPH	Ajithabai et al. (2011)
Bacopa monnieri L.	Whole plant	80% methanol	DPPH, FRAP total antioxidant	Guleria et al. (2013a)
Boerhavia diffusa L.				
Acanthospermum hispidum DC	Whole plant	Petroleum ether (60–80 °C), chloroform, acetone, ethanol, water	H_2O_2, NO$^{\bullet}$, phosphomolybdate assay, reducing power	Gomathi et al. (2013)
Adiantum lunulatum	Whole plant	50% ethanol	DPPH, H_2O_2, NO$^{\bullet}$, $^{\bullet}OH$, reducing power	Sawant et al. (2009)
Allium cepa	Bulbs	Water	DPPH, $^{\bullet}O_2^-$,$^{\bullet}OH$	Kumar et al. (2013)
		Hexane, ethyl acetate, ethanol	DPPH, $ABTS^{\bullet+}$	Fidrianny et al. (2013)
Allium sativum	Bulbs	Hexane, ethyl acetate, ethanol	DPPH, $ABTS^{\bullet+}$	Fidrianny et al. (2013)
Amorphophallus campanulatus (Roxb.) Blume. ex Decne	Tubers	Methanol, water	DPPH, NO$^{\bullet}$, reducing power	Sahu et al. (2009)
Anethum sowa	Whole plant	Water	DPPH, NO$^{\bullet}$	Kumar et al. (2011a, b)
Pentanema vestitum L.	Whole plant	70% methanol	DPPH	Ilahi et al. (2013)
Eclipta alba	Whole plant	Ethanol	DPPH, $ABTS^{\bullet+}$, NO$^{\bullet}$, phosphomolybdate assay	Baldi et al. (2011)

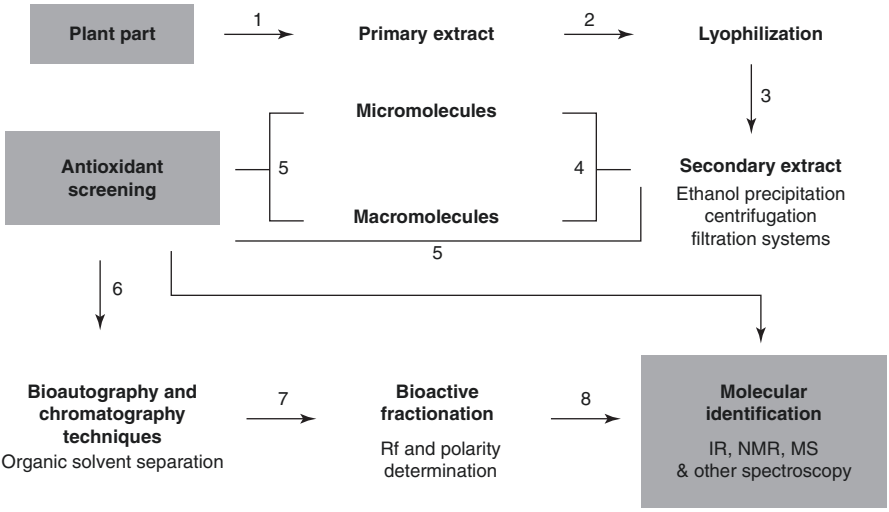
Diplazium esculentum (Koenig ex Retz.) Sw.	Young fronds	70% methanol	$ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^1O_2$, H_2O_2, $^{\bullet}OH$, $NO^{\bullet}$, $ONOO^-$, HOCl, lipid peroxidation, iron chelation, reducing power	Roy et al. (2013)
Gymnema sylvestre R.	Leaves	70% methanol	$ABTS^{\bullet+}$, DPPH,$^{\bullet}O_2^-$, $^1O_2$, $^{\bullet}OH$, $NO^{\bullet}$, $ONOO^-$, HOCl, lipid peroxidation, iron chelation, DNA protection	Sarkar et al. (2009b)
Ocimum sanctum L.	Leaves	98% methanol	DPPH, FTC, TBA, total antioxidant	Aqil et al. (2006)
Centella asiatica	Leaves	Methanol	DPPH, $NO^{\bullet}$, reducing power	Chippada and Vangalapati (2011)
Aloe vera (L.) Burm f.	Leaves	80% methanol	DPPH, FRAP total antioxidant	Guleria et al. (2013a)
Acanthus ilicifolius	Leaves	70% ethanol	$^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, lipid peroxidation	Babu et al. (2001)
	Flowers	Acetone, methanol, 70% acetone, 80% methanol, water	DPPH	Firdaus et al. (2013)
Adiantum philippense L.	Leaves	Methanol	DPPH, reducing power	Ali et al. (2013)
Andrographis paniculata	Leaves	Petroleum ether, ethyl acetate, ethanol, hydroalcohol	DPPH, $^{\bullet}O_2^-$, $^{\bullet}OH$, $NO^{\bullet}$, reducing power	Saranya et al. (2010)
Apium graveolens L.	Leaves	Ethyl acetate, methanol, butanol, water	DPPH, β-carotene-linoleate antioxidant assay	Jung et al. (2011)
Trichosanthes dioica Roxb.	Arial part (leaves, stem)	Petroleum ether, ethyl acetate, methanol, water	$NO^{\bullet}$, phosphomolybdate assay	Akter et al. (2011)
	Fruits	Water	DPPH, H_2O_2, $NO^{\bullet}$, reducing power	Shivhare et al. (2009)
Rubia cordifolia	Leaves	Hexane, chloroform, methanol	DPPH,$^{\bullet}O_2^-$, $NO^{\bullet}$	Prajapati and Parmar (2011)

(continued)

Table 2.3 (continued)

Name of the herb	Herb part used	Solvent used for extraction	In vitro antioxidant and radical scavenging assays/in vivo experiments	References
Paederia foetida	Leaves	70% ethanol	DPPH, $NO^{\bullet}$, H_2O_2, phosphomolybdate assay, reducing power	Uddin et al. (2014)
Tagetes patula L.	Flowers	Methanol	DPPH, $ABTS^{\bullet+}$, $^{\bullet}OH$, lipid peroxidation, reducing power	Bhattacharyya et al. (2010)
Blepharis molluginifolia	Flowers	Petroleum ether, benzene, chloroform, acetone, water, ethanol, methanol	DPPH, $ABTS^{\bullet+}$, H_2O_2	Deepika and Rajagopal (2014)
Tropaeolum majus L.	Leaves and flowers	Water, ethanol	DPPH, $^{\bullet}O_2^-$, H_2O_2	Bazylko et al. (2014)
Opuntia ficus indica f. *inermis*	Flowers	50% methanol	DPPH, FRAP total antioxidant, linoleic acid peroxidation, levels of CAT, SOD, lipid peroxidation	Alimi et al. (2011)
Tagetes erecta L.	Flowers	1% HCL in methanol	DPPH, FRAP total antioxidant	Siriamornpun et al. (2012)
Alpinia zerumbet (Pers.) B. L. Burtt. and R. M. Sm.	Flowers	Ethyl acetate	DPPH, β-carotene antioxidant assay	Elzaawely et al. (2007)
	Seeds			
Melastoma malabathricum L.	Flowers	Hexane, ethyl acetate, methanol	DPPH	Susanti et al. (2007)
Nelumbo nucifera	Seeds	50% ethanol	DPPH, $NO^{\bullet}$, levels of CAT, SOD, lipid peroxidation	Rai et al. (2006)
Macrotyloma uniflorum (Lam.) Verdc	Seeds	Methanol, 70% acetone	DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^{\bullet}OH$, FRAP total antioxidant, linoleic acid antioxidant assay	Siddhuraju and Manian (2007)

Vigna unguiculata (L.) Walp.	Seeds	70% acetone	DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^{\bullet}OH$, FRAP total antioxidant, β-carotene/linoleic acid antioxidant assay	Siddhuraju and Becker (2007)
Vigna aconitifolia (Jacq.)	Seeds	70% acetone	DPPH, $ABTS^{\bullet+}$, $^{\bullet}O_2^-$, $^{\bullet}OH$, FRAP total antioxidant, linoleic acid antioxidant assay, iron chelation	Siddhuraju (2006)
Piper cubeba L.	Seeds	98% methanol	DPPH, FTC, TBA, total antioxidant	Aqil et al. (2006)
Coriandrum sativum L.	Fruits	80% methanol	DPPH, FRAP total antioxidant	Guleria et al. (2013a)
	Leaves	95% ethanol	Reducing power	Sharma and Shrivastava (2013)
Celastrus paniculatus Willd.	Seeds	80% methanol	DPPH, FRAP total antioxidant	Guleria et al. (2013a)
		Petroleum ether, ethyl acetate, methanol, water	DPPH, $NO^{\bullet}$, phosphomolybdate assay, cupric reducing antioxidant capacity, reducing power	Zohera et al. (2010)
Ammi visnaga	Fruits	Ethanol, water	DPPH, iron chelation	Hilmi et al. (2014)
Nigella sativa	Seeds	Hexane, chloroform, ethyl acetate, methanol, water	DPPH, $^{\bullet}OH$, iron chelation, β-carotene antioxidant assay	Meziti et al. (2012)
		Ethanol, water	DPPH, iron chelation	Hilmi et al. (2014)
Citrullus lanatus	Seeds	Hexane, chloroform, ethanol	DPPH, $NO^{\bullet}$, H_2O_2, reducing power	Rahman et al. (2013)
Tribulus terrestris	Fruits	Hexane, water	DPPH, $^{\bullet}O_2^-$, $NO^{\bullet}$, FRAP total antioxidant, iron chelation	Bhat et al. (2012)
Citrullus colocynthis (L) Schrad.	Fruits	Methanol	DPPH, $^{\bullet}O_2^-$, H_2O_2, $^{\bullet}OH$, $NO^{\bullet}$	Kumar et al. (2008)



2.6 Conclusions

The Indian subcontinent is one of the richest ecosystems in the world with a great variety of plant species with antioxidant compounds of known and unknown nature. There has been growing interest in medicines derived from plants because of their minimal or no toxicity, negligible side-effects, ease of incorporation in the health system due to their biological origins, ease of procurement and low manufacturing and trading costs. Despite enormous interest in the therapeutic uses of medicinal plants, scientists face obstacles related to their identification, medical effectiveness, therapeutic dosage, toxicity, standardization and regulation.

The urge to discover novel plant compounds with antioxidant activity for healthcare and disease prevention is now an essential ingredient of contemporary pharmaceutical research. With rapidly growing demand for medicinal plants, a reasoned and sustainable exploitation of the flora in nature is more than important to apply. Indeed, the rapid loss of forests and restricted opportunities in botany and medicinal chemistry in university curricula will be limiting factors in the search for plant-based therapeutics that can offer promise for prevention and treatment of chronic diseases. A thorough characterization of bioactive plant compounds is essential for their acceptance into mainstream medicinal practice. Identification of phytocompounds (Fig. 2.3) provides unlimited opportunities for alternative and new preventive healthcare and therapeutic strategies against NCDs.

Fig. 2.3 Standardization flowchart from extraction to identification of bioactive phytocompounds. (*1*) Plants are chosen either randomly based on literature reports or after consultation with local healers and then followed by botanical identification. (*2*) Collected plant material is ground to optimize the solvent contact during the extraction process. Weight standardization is necessary (i.e. 100 g of plant material to 1000 ml of solvent). The primary extraction methods are variable, but the goal is to investigate reports of popular use and apply similar extraction methods. (*3*) After extraction, the volume is concentrated by lyophilization or using another concentration technique before screening. Lyophilization produces ground powder which is then resuspended in water for initial screening to confirm bioactivity, if present. (*4*) Due to the complex composition of the extract, primary separation can be used to facilitate the identification process. Micromolecules can be separated from macromolecules (proteins and carbohydrates) from the supernatant and precipitate phases obtained (*5*). The antioxidant screening by evaluation of free radical scavenging activities is the most efficient and inexpensive assay to identify initial bioactivity. (*6*) Bio-guided chromatography techniques such as bioautography preceded by solvent separation are essential to initiate the bioactive phytocompound identification process; fraction collection with high-performance liquid chromatography (HPLC) or fast protein liquid chromatography (FPLC) assays and preparative thin-layer chromatography (TLC) are also valid techniques. Bio-guided fraction and purification confirm previous results and lead to isolation of a bioactive phytocompound. (*7*) By using TLC assays, retention factor (Rf) values can be determined and the polarity or even chemical groups (by using specific dyes) elucidated. (*8*) Nuclear magnetic resonance (NMR), HPLC/mass spectrometry (MS) and gas chromatography/mass spectrometry (GC/MS) are used to identify bioactive phytocompounds

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Chapter 3
Antioxidant Potential of African Medicinal Plants

Ashwell Rungano Ndhlala, Bhekumthetho Ncube, Hafiz Ahmed Abdelgadir, Christian Phillipus Du Plooy, and Johannes Van Staden

Abstract In Africa, the use of medicinal plants to combat diseases forms an integral component of the culture and is thus practised widely. To this extent, several important plant species have been subjected to different pharmacological screening techniques to give scientific credence to the claimed uses. Among those that have been screened are those used to combat oxidative stress. We present here a review of the important plants used in African traditional medicine with antioxidant potential. The plants listed here are habitats of North Africa, covering the vast Sahara, West Africa, East Africa and the Palaeotropical kingdom of Central Africa as well as Southern Africa including the Capensis kingdom of the Western Cape province of South Africa, Madagascar, Mauritius and the Mascarenes. Oxidative stress has been implicated as one of the key factors in accelerated pathogenesis of a number of human diseases including cardiovascular, inflammatory, cancer, autoimmune and neurodegenerative diseases. Plants listed here have been utilised directly or indirectly in managements of some of these conditions.

Keywords African medicinal plants • Antioxidants • Reactive oxygen species • Health benefits • Disease prevention

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3.1 Introduction

The research and discovery of antioxidants date back to antiquity (Gutteridge and Halliwell 2010). To date the subject has attracted considerable attention from researchers and millions of resources in funding. Furthermore, numerous studies have ascribed several compounds to be beneficiary mainly because they possess antioxidant properties. Antioxidants are substances that, at low concentrations, prevent or retard the oxidation of biomolecules such as lipids, proteins and nucleic acids (Becker et al. 2004). The oxidation of these important molecules is mediated by reactive oxygen species (ROS), which are highly reactive, short-lived molecules with one or more unpaired electrons. In many instances, ROS are a result of mitochondrial respiration (Tahara et al. 2009; Watson 2013), and in other ways, they represent toxic by-products of a variety of pathways, including both enzyme-catalysed reactions and non-enzymatic reactions. Most ROS are generated through the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX enzymes) (Lambeth et al. 2008). In some cases, free radicals are produced in response to cellular damage as a way to signal the body's own repair mechanisms. Living cells have evolved mechanistic systems, which operate as antioxidants by delaying or preventing oxidative cell damage from ROS (Vurusaner et al. 2011).

Antioxidants and ROS are involved in a number of processes within the living cell, permeating the whole of life and have resulted in the fascinating discipline of redox/systems biology. Normal cell functioning thus depends on a balance between the two. Like a tug-of-war, the antioxidants perform their biochemical roles by decreasing the levels of free radicals thereby allowing for a reduced molecular damage (Ndhlala et al. 2014a). However, in some instances, damage becomes inevitable and so will be oxidative stress, which then require repair systems or supplementary/natural antioxidants to maintain cell viability. The most abundant natural antioxidants are either plant-derived water-soluble substances such as vitamin C and phenolic compounds or lipid-soluble vitamin E and carotenoids.

In this chapter, information on important medicinal plants used traditionally as antioxidant agents and which have been scientifically investigated for antioxidant potential was gathered. The information gathered was largely distributed among two regions of Africa, North Africa (represented by the vast Sahara, West Africa, East Africa and Central Africa geographical zones) and Southern Africa (represented by countries to the south of the equator including Madagascar, Mauritius and the Mascarenes). With only a few exceptions, Africa's flora is either tropical or subtropical. Plants found in these regions include savanna grasses and herbs, as well as savanna shrubs and trees. Tropical forests make up a much smaller area of Africa being mostly abundant in portions of Central Africa (Brendler et al. 2010).

3.2 Medicinal Plants in Africa

An estimated 80% of the African population depends on traditional medicine, and its significance and impact among these populations in the continent cannot be ignored (WHO 2005). The reliance of such a large proportion of these populations on traditional medicine is mainly because of its availability, accessibility and affordability. In Africa, there exist a pool of extensive traditional knowledge and expertise within the communities (Fennell et al. 2004). Africa is bestowed with two characteristic floral kingdoms: the Palaeotropical kingdom of Central Africa and the Capensis kingdom of the Western Cape province of South Africa, the latter of which contains an estimated 10,000 species, representing roughly 20% and 10% of Africa's and the world's floral biodiversity, respectively (Ernst-Detlef et al. 2005; Ndhlala et al. 2013a). The difference in the plant distribution is mainly because of the variations in the rainfall patterns across Africa. The southern tip together with the northwest part of Africa is characterised by winter rainfall. The Southern African tip is the traditional home of the Khoi/San whose history is rich with knowledge and expertise in medicinal plant usage. The Arab medicine, which is practised in the northeast parts of Africa, is also an important aspect of the traditions of Africa. Arab medicine is, however, greatly influenced by the Asian and Greek scientific as well as the philosophical works (Ndhlala et al. 2013a).

3.3 Natural Antioxidants: Sources and Uses

The discovery of the role of free radicals in cancer, diabetes, cardiovascular diseases, autoimmune diseases and neurodegenerative disorders and their implication in ageing and other diseases has necessitated a medical revolution with promising potential for healthcare. The inherent human antioxidant defence systems comprise enzymatic scavengers like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). In addition, enzymes involved in the reduction of oxidised forms of molecular antioxidants like glutathione reductase (GR) and dehydroascorbate reductase (DHAR) also form part of this diverse system (Ratnam et al. 2006). Apart from scavengers, there exists cellular machinery, which maintains a reducing environment, for example, regeneration of NADPH by glucose-6-phosphate dehydrogenase. These systems work together as a network in scavenging ROS, trapping of the singlet oxygen, chelating of harmful metal ions, regenerating 'spent' antioxidants, stimulating other antioxidant systems, inhibiting lipid peroxidation, reducing mitochondrial oxidative stress, inhibiting DNA-carcinogen adduct formation, suppressing free radical production and upregulating other antioxidant enzymes (Ndhlala et al. 2014b). However, due to the constant

exposure to external free radical sources in modern-day living, and in cases such as disease, the inherent mechanisms are not always sufficient, and supplementation with external antioxidants becomes essential. Natural antioxidants present in the diet or as supplements increase the resistance towards oxidative damages and have a substantial impact on human health. Dietary antioxidants comprise a group of several different compounds, mainly secondary metabolites that plants synthesise to protect themselves against oxidative stress. The nutritional and health benefits of plant sources as antioxidants are largely ascribed to these diverse chemical species, with multiple functional biological properties. Based on their chemistry, dietary antioxidants may be grouped into four types: vitamin C (ascorbic acid), vitamin E (tocopherols), carotenoids (e.g. α- and β-carotenes, lycopene, lutein) and polyphenolic antioxidants (Catoni et al. 2008). The polarity of these chemical species affects their cellular level distribution in the human body system, with hydrophilic antioxidants found in the cytoplasm, while lipophilic ones are confined in cellular membranes.

Numerous studies posit the consumption of fruits and vegetables as correlating with reduced risk of degenerative diseases and are considered to be among the major dietary sources of antioxidants. Epidemiological studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk of cardiovascular diseases and certain forms of cancer (Hughes 2000; Bazzano et al. 2001). Apart from the well-known and extensively studied antioxidants like vitamins (ascorbic acid and α-tocopherol), polyphenolic compounds have recently attracted enormous research attention as promising major sources of antioxidants. Owing to their chemical diversity, distribution and abundance in the plant kingdom, polyphenolic compounds hold considerable potential as potent antioxidant agents, with numerous structures being established within plant sources. These heterogeneous compounds range from simple phenols, phenolic acids (both benzoic and cinnamic acid derivatives), coumarins, flavonoids and stilbenes to highly polymerised compounds like lignins, melanins and hydrolysable and condensed tannins (Naczk and Shahidi 2004; Lapidot et al. 1999). Among these, flavonoids have been identified as the predominant and potent components in most plant sources. Plants used in traditional medicines are one of the least exploited potential sources of antioxidants, either for direct consumption or in the production of food supplements to enrich the nutritional value and prophylaxis or therapy of certain diseases. Traditionally, herbal medicines with antioxidant properties have been used for various purposes, and epidemiological data also points at widespread acceptance and use of these agents (Rice-Evans et al. 1997; Youkeu 2008; Ndhlala et al. 2014b). Bestowed with a richly diversified natural flora, coupled with an ancient-dating culture of the use of traditional medicine that spans over generations, Africa offers vast potential in the global healthcare through medicinal plants. Table 3.1 illustrates some of the natural dietary sources of different antioxidant agents including a few examples of African medicinal plants consumed as foods.

Table 3.1 Examples of natural plant-derived sources of antioxidants

Antioxidant	Source	Reference
Flavanols	Fruits (e.g. berries, citrus fruits, grapes), parsley, beans; *Moringa oleifera* (medicinal plant—Africa)	Yanishlieva-Maslarova and Heinonen (2001); Siddhuraju and Becker (2003); Manach et al. (2004)
Hydroxycinammic acids	Berries, cherries, plums, prunes, apples, pears, kiwi, most vegetables, red wine, coffee	Manach et al. (2004); Belitz and Grosch (1999); Yanishlieva-Maslarova and Heinonen (2001)
Anthocyanins	Fruits (e.g. cherries, plums, berries, apples, oranges), vegetables (e.g. rhubarb)	Hakkinen et al. (1998); Manach et al. (2004)
Catechins	Fruits (e.g. strawberry, citrus, kiwi, plums, prunes, apples, pears)	Belitz and Grosch (1999)
Flavonols	Vegetables (e.g. parsley, kale, spinach, leek, sweet potato leaves, beans), black and green tea, black grapes, *Moringa oleifera* (medicinal plant used across Africa)	Chu et al. (2000); Siddhuraju and Becker (2003); Manach et al. (2004)
Rosmarinic acid	Herbs and spices (e.g. rosemary, summer savoury, sage, oregano)	Yanishlieva-Maslarova and Heinonen (2001); Zheng and Wang (2001)
Ascorbic acid (vitamin C)	Fruits and vegetables (tomatoes, strawberry, citrus, kiwi, Brussels sprout, cauliflower, green vegetables), *Mondia whitei* and *Moringa oleifera* (medicinal plants used across Africa)	Lindsay and Clifford (2000); Szeto et al. (2002); Siddhuraju and Becker (2003); Youkeu (2008); Oketch-Rabah (2012)
Vitamin E (α, β, δ, γ isomers of tocopherols and tocotrienols)	Green vegetables, gettable oils, seeds, sunflower, nuts, wheat, *Moringa oleifera* (medicinal plant—Africa)	Yanishlieva-Maslarova and Heinonen (2001); Szeto et al. (2002); Anwar et al. (2007)
Carotenoids	Orange-/red-coloured fruits and vegetables (melon, carrot, tomato, apricot, peach, broccoli, leafy vegetables)	Belitz and Grosch (1999); Manach et al. (2004)
Quercitrin, 6,8-diprenyleridictyol	*Dorstenia picta* (medicinal plant—Cameroon)	Omisore et al. (2004, 2005)
Oleuropein, verbascoside	*Olea europaea* (medicinal plant used in Tunisia)	Dekanski et al. (2009)
(-)-loliolide, 2-hydroxy-4-methoxybenzaldehyde, chlorinated coumarinolignan	*Mondia whitei* (medicinal plant used across Africa)	Kubo and Kinst-Hori (1999); Patnam et al. (2005); Neergaard et al. (2010)
Quercetin, kaempferol	*Moringa oleifera* (medicinal plant used across Africa)	Siddhuraju and Becker (2003); Anwar et al. (2007)

3.4 Important African Medicinal Plants with Antioxidant Potential

Many of the benefits derived from intake of crude extracts of most traditional herbal remedies are attributable either to specific chemical constituents or synergistic interactions among a pool of phytochemical compounds. Literature is replete with information detailing significant successes and potential in the prophylactic and therapeutic effects of African medicinal plant extracts in reducing neurodegenerative and cardiovascular diseases, diabetes, inflammatory diseases, cancer, ageing and various other oxidative disorders. Table 3.2 illustrates a few examples of African medicinal plants with antioxidant potential. The majority of the plants in Table 3.2 are used traditionally for treating various ailments, but they all have one thing in common, which is the ability to alleviate oxidative stress. Among these plants, several are distributed throughout Africa, while some were introduced into some sections of Africa, and some are restricted. Those that are distributed across Africa include *Amaranthus hybridus*, *Croton gratissimus*, *Cajanus cajan*, *Urtica dioica*, *Carissa edulis*, *Diospyros abyssinica* and *Pelargonium sidoides*. Species such as *Adansonia digitata* and *Moringa oleifera* have been successfully introduced in the tropical savanna and subtropical desert of the continent.

Although, historically, *Pelargonium sidoides* (Fig. 3.1d and Table 3.2) has been used by African indigenous tribes particularly in South Africa to treat various disorders such as gastrointestinal disorders and respiratory tract infections, the global interest in the herbal drug has seen a proprietary extract of the roots of the plant, EPs® 7630 being developed as a modern phytopharmaceutical drug. Among other positive therapeutic effects, the drug has demonstrated significantly strong immunomodulatory and secretomotoric effects. Although, identification of individual chemical constituents responsible for specific pharmacological properties are yet to be identified, the efficacy of *P. sidoides* has been attributed to the activity of highly oxygenated coumarins (7-hydroxy-5,6-dimethoxycoumarin; 6,8-dihydroxy-5,7-dimethoxycoumarin), gallic acid derivatives, flavonoids and hydroxycinnamic acid derivatives (Kolodziej 2007; Colling et al. 2010), all of which are polyphenolic compounds with established antioxidant properties (Rice-Evans et al. 1997). In addition, the chemical components of the ethanolic root extract of the plant consist largely of oligo- and polymeric proanthocyanidins, which are based on gallocatechin and epigallocatechin moieties (Theisen and Muller 2012). Coumarins and phenolic compounds including simple phenolic acids and proanthocyanidins are the principal compounds found in the special extract, EPs® 7630, with gallic acid consistently found in fairly high concentrations.

Compared to standard antioxidant like butylated hydroxyanisole (BHA), the alcoholic (80% ethanol and methanol) root bark extracts of *Diospyros abyssinica* exhibited significantly higher radical scavenging activity when tested for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay (Maiga et al. 2006). Of particular interest in this positive antioxidant activity is that 36.7% of the plant material weight was found to be composed of antioxidants,

Table 3.2 Some important African medicinal plants with antioxidant potential

Plant family	Plant name	Traditional uses and distribution in Africa	Reported pharmacological activities	Active components	References
Amaranthaceae	*Amaranthus hybridus* L.	Leafy vegetable, cereal grain (seed), used to treat diarrhoea; leaf poultice is used to treat bee, wasp, hornet and scorpion stings and snake and insect bites. Leaf infusions are used as mouthwash. The leaves encourage blood clotting and are used in folk medicine against nose bleeding. A strong leaf decoction is used to remove worms and other parasites from the digestive tract	Antioxidant, astringent, haemostatic, antidiarrhoeal, nutritive, alterative, diuretic, alkalizing and anthelmintic properties	Caffeic acid, protocatechuic acid and ferulic acid	Akubugwo et al. (2007); Chitindingu et al. (2007); Odhav et al. (2007)
Asclepiadaceae	*Solenostemma arghel* (Del.) Hayne	Used as a remedy for rheumatic pain, cough and cold and in cardiovascular diseases. The plant is native in tropical Africa, spreading across the central Sahara to the Sinai and the south-eastern (Arabian) desert. Also used in Algeria, Libya and Egypt	Antioxidant and antimicrobial activity	Argelin, argelosid, choline, quercetin and kaempferol	Al-Jaber et al. (2011)
Brassicaceae	*Sisymbrium erysimoides* Desf.	Used in the treatment of cough and bronchial disorders. Leaf infusion is an effective remedy of sore throat. The plant is native to Northern Africa mainly Algeria, Egypt, Libya, Morocco and Tunisia	Antioxidant, anti-inflammatory and analgesic activities	Apigenin, apigenin-7-*O*-b-rhamnoside, kampferol-3-xyloside-7-galactoside, quercetin-6,40-dimethoxy-3-fructorhamnoside, quercetin 40-methoxy-3-fructo-rhamnoside	Cook et al. (1998); Al-Jaber et al. (2011)

(continued)

Table 3.2 (continued)

Plant family	Plant name	Traditional uses and distribution in Africa	Reported pharmacological activities	Active components	References
Burseraceae	*Commiphora myrrha* (Nees) Engl.	Used in ancient times in Egypt for preserving bodies. In Somalia and Ethiopia, the plant gum-resin is used to treat stomachache. Commercial uses include additive in cosmetics, skin lotions, bath soaps, mouthwashes and air fresheners	Analgesic, antibacterial, and antifungal activities. Work as a local anaesthetic	Furanoeudesma-1,3-diene, sesquiterpene and curzerene	Brendler et al. (2010); Stoilova et al. (2014)
Guttiferae	*Garcinia kola* Heckel	The seeds are used for bronchitis, throat infections, colic, head or chest colds and cough. It is also used for liver disorders. The bark is used for the treatment of malignant tumours	Antioxidant, purgative, anti-inflammatory, antidiabetic, antiparasitic, antiviral and antimicrobial activities	Kolanone, eridictoyl/taxifolin, garcifurans A and B, garcinoic acid	Brendler et al. (2010); Farombi et al. (2002)
Compositae	*Pluchea Arabica* (Boiss) Qaiser and Lack	Commonly used in North African countries to reduce swelling and to treat boils	Antioxidant activity	Quercetin, sesquiterpenes (godotol A and B)	Al-Jaber et al. (2011)
Cucurbitaceae	*Momordica charantia* L.	Used in Morocco and Egypt as an antidiabetic agent	Antioxidant	Methoxybenzoic acid, momordicoside	Virdi et al. (2003); Wu and Ng (2008); Nafiu et al. (2013)
Euphorbiaceae	*Croton gratissimus* Burch.	Used in Nigeria and Southern Africa to treat coughs, fever, abdominal disorders, respiratory disorders, skin inflammation, earache, malarial and chest complaints	Antiplasmodial, antioxidant, cholinesterase inhibitory effects	Cembranolides, kaempferol-3-*O*-β-6″(*p*-coumaroyl) glucopyranoside (tiliroside), apigenin-6-*C*-glucoside (isovitexin) and kaempferol	Ndhlala et al. (2013b)

Euphorbiaceae	*Mallotus oppositifolius* (Geiseler) Müll.Arg.	Used in Nigeria for anaemia and pneumonia and as an aphrodisiac, antimalarial as well as inflammation agent	Anti-inflammatory, antimicrobial, antiproliferative, antioxidant and antiplasmodial activities	Mallotojaponins B and C, mallotophenone	Farombi et al. (2002); Nafiu et al. (2013)
Humiriaceae	*Sacoglottis gabonensis* Baill.	Used for abdominal pain, fever, gonorrhoea, hypertension, diarrhoea and diabetes. Commonly found in Senegal, Gambia, and Central African Republic	Antioxidant activity, hepatoprotective and antilipid peroxidation properties	Bergenin	Maduka et al. (2004); Nafiu et al. (2013)
Leguminosae	*Cajanus cajan* (L.) Millsp.	Used in traditional medicine for anaemia, hepatitis, diabetes and urinary tract infections. The plant grows throughout Africa	Antioxidant, antimalarial and antisickling activities	Cajaninstilbene acid, pinostrobin, vitexin and cajanol	Wu et al. (2008); Brendler et al. (2010)
Leguminosae	*Alhagi maurorum* Boiss	Native to North Africa including Algeria, Egypt, Libya, Niger and Sudan, occurring along the Nile region including the Delta valley, the plant is used for rheumatic pain, bilharzia, liver disorders, urinary tract infections and gastrointestinal disorders	Antioxidant and antiulcerogenic activity	Tamarixetin 3-*O*-dirhamnoside, quercetin 3-*O*-rhamnoside, isorhamnetin	Al-Jaber et al. (2011)

(continued)

Table 3.2 (continued)

Plant family	Plant name	Traditional uses and distribution in Africa	Reported pharmacological activities	Active components	References
Malvaceae	*Adansonia digitata* AL.	The fruit pulp is used as an immunostimulant, in the treatment of diarrhoea, dysentery and hiccough. The oil from the seeds is used for inflamed gums and toothache. In East Africa, it is used as an antidote to *Strophanthus* poisoning. Introduced in most parts of Africa, mostly south of the Sahara region	Antioxidant, anti-inflammation, antimicrobial and antipyretic activities	Ascorbic acid, campesterol, isofucasterol, avenasterol, lupeol, scopoletin, friedelin and tocopherols	Brendler et al. (2010)
Moraceae	*Dorstenia picta* Bureau	Used in Cameroon for diabetes, headache, and hypertension	Antioxidant, antihypertensive, anti-inflammation, antimicrobial and antinociceptive activities	Quercitrin, 6,8-diprenyleridictyol	Kanscie et al. (2003); Omisore et al. (2005)
Oleaceae	*Olea europaea* L.	Used for fever, hypertension and diabetics in Tunisia	Antioxidant, antimicrobial, antiviral, antitumour activities	Oleside, oleuropein, verbascoside	Dekanski et al. (2009); Nafiu et al. (2013)
Urticaceae	*Urtica dioica* L.	Used in traditional medicine to ease allergy symptoms, particularly hay fever. Found in many parts of DRC, Nigeria, Tanzania and Sudan	Antioxidant and antihistamine activities	Antihistamines	Yesilada et al. (1997)

Apocynaceae	*Carissa edulis* (Forssk.) Vahl	Used traditionally in South Africa, Botswana, Namibia, Kenya, Tanzania, Nigeria, Uganda and Senegal to treat venereal diseases, leukaemia, diabetes, fever, diarrhoea, stomach problems, sickle cell anaemia, headache, chest complains, rheumatism, oedema, gonorrhoea, syphilis, rabies, fever, cough, ulcer, toothache and worm infestation	Antioxidant, antimicrobial, antidiabetic, antiviral, anticonvulsant, amoebicide, cardiotonic, antiplasmodial, hypoglycaemic and antileukaemic activities	4-hydroxy-(3-hydroxypropionyl) benzene, coniferaldehyde, quebrachitol, catalponol, 2-hydroxyacetophenone, quercetin-3-*O*-β-D-glucopyranoside, rhamnetin-3-*O*-β-D-glucopyranoside, isorhamnetin-3-*O*-β-D-glucopyranoside	Ndamba et al. (1994); Tolo et al. (2006); Quang (2010); Al-Youssef and Hassan (2017)
Bignoniaceae	*Newbouldia laevis* Seem.	Roots are used to cure migraine, earache and stomachache, while the leaves are used to combat eye disease and breast cancer and the stem barks to treat dysentery, rheumatoid arthritis, epilepsy and skin infections. Found in the southern	Antioxidant, antimicrobial, anthelmintic, anti-inflammation, analgesic activities, immunomodulatory effects	Verbascoside, 2,3-dehydrofuranonaphthoquinones, 2-acetyl-5-hydroxynaphtho[2,3-*b*] furan-4,9-dione, 2-isopropenylnaphtho[2,3-*b*] furan-4,9-dione; 5-hydroxy-dehydroiso-a-lapachone; martynoside	Gafner et al. (1998); Houghton et al. (1994); Gormanna et al. (2003)
Asphodelaceae	*Bulbine capitata* Poelln.	Distributed in Southern Africa but occurs mostly in Botswana. Used for the treatment of fungal and bacterial infections, skin rush, wounds, burns	Antioxidant, antibacterial, antiviral, antifungal activities	2-hydroxy-3-methoxy-5-(2-propenyl) phenol, foliosone, knipholone, chrysophanol, apigenin, luteolin, 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-c] furan-4,9-dione	Achenbach et al. (1983); Bezabih et al. (1997); Bringmann et al. (1999)

(continued)

Table 3.2 (continued)

Plant family	Plant name	Traditional uses and distribution in Africa	Reported pharmacological activities	Active components	References
Ebenaceae	*Diospyros abyssinica* (Hiern) F. White	Every part of the plant has been used traditionally in Africa as an astringent and remedy for snake bites and to cure biliousness. It grows in the southern part of Africa but also found in Angola, Guinea, Eritrea and Ethiopia	Antioxidant, antimycobacterial and antimicrobial activities	Betulin, betulinic acid, momordicoside, *p*-methoxybenzoic acid, oleuropein	Mallavadhani et al. (1998); Moghaddam et al. (2012); Maiga et al. (2006)
Geraniaceae	*Pelargonium sidoides* DC	Used by African indigenous tribes as a traditional medicine for curing various ailments, including diarrhoea, colic, gastritis, tuberculosis, dysentery, cough, hepatic disorders, skin pimples, menstrual complaints and gonorrhoea. Largely distributed in the southern parts of Africa	Antimicrobial, antiviral, immunomodulatory, anthelmintic and antioxidant activities	Dihydroquercetin (taxifolin), *p*-hydroxyphenylethanol, *p*-coumaroyl-4-*O*-b-D-glucoside, scopoletin, umckalin, 6,8-dihydroxy-5,7-dimethoxycoumarin, fraxetin, phyllantusiin E, orientin, isovitexin, taxifolin-3-*O*-b-D-glucoside, 6,8-dihydroxy-5,7-dimethoxycoumarin	Kolodziej (2007); Brendler and Van Wyk (2008); Colling et al. (2010); Patiroglu et al. (2012); Moyo and Van Staden (2014)
Bignoniaceae	*Kigelia africana* (Lam.) Benth	Used as purgative and in dysentery, haemorrhoids, constipation, wounds, ulcers, boils, liver diseases, abscesses, rheumatism, syphilis, gonorrhoea, emollient, antieczema and skin-firming properties. A true multipurpose tree found mostly in the subtropical regions of Africa	Antioxidant, antimicrobial, antiprotozoal, anti-inflammatory activities, androgenic effect, antipsoriasis	γ-Sitosterol, campesterol, verminoside, verbascoside, caffeic acid, *p*-coumaric acid, luteolin, 6-hydroxyluteriol, dihydroisocoumarine kigelin, lapachol, isopinnatal, kigelinol, isokigelinol	Khana and Mlungwana (1998); Patrizia et al. (2005); Olaleyea and Rocha (2007, 2008); Azu et al. (2010)

Fabaceae	*Sutherlandia frutescens* L. R. (Br.)	Use in Southern Africa to treat indigestion, stomach complaints, dysentery, colds, influenza, kidney conditions, fever, diabetes, internal cancers, uterine troubles, liver conditions, backache, rheumatoid arthritis, urinary tract infections, stress and anxiety, dropsy and heart failure. It is indigenous to South Africa, Lesotho, southern Namibia and south-eastern Botswana	Antioxidant, anticarcinogenic, anticonvulsant, antithrombotic, anticancer, anti-inflammatory, antiviral, anti-HIV, antimicrobial, antistress and antidiabetic activities	Sutherlandiosides, flavonoid glycosides, pinitol	Van Wyk and Albrecht (2008); Avula et al. (2010); Faleschini et al. (2013); Williams et al. (2013)
Apocynaceae	*Mondia whitei* (Hook.f.) Skeels	The various parts are used for the management of several cases, among them are impotence, sexual dysfunction, constipation, abdominal pain, appetite stimulant, stress, tension, urinary infections and gonorrhoea and as analgesic pains and inducement of labour. It is endemic to South, East and West Africa.	Androgenic effect, anthelmintic, aphrodisiac, antimicrobial, anti-inflammatory, anti-tyrosinase and antioxidant activities	2-hydroxy-4-methoxybenzaldehyde, chlorinated coumarinolignan, propacin, loliolide, isovanillin, β-sitosterol	Kubo and Kinst-Hori (1999); Matu and Van Staden (2003); Patnam et al. (2005); Abdou Bouba et al. (2010)

(continued)

Table 3.2 (continued)

Plant family	Plant name	Traditional uses and distribution in Africa	Reported pharmacological activities	Active components	References
Moringaceae	*Moringa oleifera* Lam	Native to the western and sub-Himalayan tracts, India, Pakistan, Africa and Arabia; various plants parts are used in the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, haematological and hepatorenal disorders	Antioxidant, antimicrobial, anti-inflammatory, antitumor and anticancer antihypertensive, diuretic, antispasmodic, antiulcer, hepatoprotective and cholesterol-lowering activities	Vanillin, β-sitosterol β-sitostenone, 4-hydroxymellin, octacosanoic acid, kaempferol, rhamnetin, isoquercitrin and kaempferitrin, *O*-ethyl-4-(α-L-rhamnosyloxy)benzyl carbamate, niazimin	Faizi et al. (1994a, b, 1995, 1998); Siddhuraju and Becker (2003); Ndhlala et al. (2014c)
Asphodelaceae	*Aloe arborescens* Mill.	Leave decoctions are used to treat wounds, burns and various skin ailments including eczema, skin irritations and bruises. Decoctions are also used to treat and prevent cancer. Endemic to the south-eastern part of Southern Africa, specifically South Africa, Malawi, Mozambique and Zimbabwe	Antioxidant, anti-inflammatory, wound healing, skin-lightening properties	Aloesin, glycoproteins	Van Wyk et al. (2009)

Fig. 3.1 Some important African medicinal plants with antioxidant properties. (**a**) Wildly growing *Aloe arborescens*. (**b**) *Kigelia africana*. (**c**) Cultivated *Artemisia annua* at the Agricultural Research Council (South Africa) experimental farm in Roodeplaat, Pretoria. (**d**) Cultivated *Pelargonium sidoides* at the University of KwaZulu-Natal, Pietermaritzburg Botanical Garden

with root barks being the richest source of the extracted compounds. The most frequently isolated compounds from *D. abyssinica* are the triterpenoids betulin, betulinic acid and lupeol. These compounds have been established as antioxidant agents (Mallavadhani et al. 1998). The plant grows mostly in the southern part of Africa, and nearly every part of the plant is used for medicinal purposes.

Indigenous to South Africa, Lesotho, southern Namibia and south-eastern Botswana, *Sutherlandia frutescens* extracts have shown antiproliferative, anti-HIV, antidiabetic, anti-inflammatory, analgesic, antibacterial, antistress, anticonvulsant and antithrombotic activities both in vitro and in vivo (Van Wyk and Albrecht 2008). Williams et al. (2013) investigated the capacity of an aqueous extract of *S. frutescens* to prevent insulin resistance (a precursor of type 2 diabetes) in a human liver cell culture and to identify genes regulated by *S. frutescens* treatment. Their results confirmed that the extract can prevent insulin resistance in hepatocytes over a 48 h period. Treatment with *S. frutescens* extracts reversed this, upregulating the majority of the diabetes-related genes indicating that the plant extract aids in maintaining normal insulin responses. The identified changes in gene expression indicate several potential mechanisms of antidiabetic action for *S. frutescens*, reflecting the multiple bioactive compounds previously identified in aqueous extracts of the plant species (Avula et al. 2010) (Table 3.2).

Numerous antioxidant compounds have been isolated from *Dorstenia picta*, a traditional medicinal plant used mostly in Cameroon in the treatment of diabetes and hypertension. The compounds include quercitrin, 6,8-diprenyleridictyol, bartericin A and 6-prenylapigenin (Omisore et al. 2005). Some of the confirmed pharmacological properties from the plant extracts include antihypertensive, antioxidant, anti-inflammatory and antinociceptive activities. Apart from their medicinal uses, members of the genus are also used in the preparation of food such as *D. foetida* and *D. psilurus* whose tubers and rhizomes are cooked and/or used as spices, respectively (Omisore et al. 2004). The fact that these plant species form part of the diet, and considering the impressive antioxidant properties displayed by their extracts, makes them ideal candidates for the development of antioxidant dietary supplements.

Native to India but now successfully introduced and indigenised well beyond its native range in Africa, *Moringa oleifera* (Fig. 3.2) is one of the promising medicinal plants with multiple uses. The plant is mainly used as a nutritious vegetable, either cooked or pickled. The tender leaves are rich in proteins, minerals, β-carotene, thiamine, riboflavin and ascorbic acid. The plant has high ascorbic acid which makes it an antioxidant agent. Several other compounds including alkaloids, flavonoids, anthocyanins, proanthocyanidins and cinnamates are distributed through the plant. Of particular importance are the glycosides, niazirin and niazirinin isolated from the leaves, 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate isolated from the roots and 4-hydroxymellein, vanillin, β-sitosterone, octacosanic acid and β-sitosterol in the stem which have antioxidant properties.

In *Olea europaea*, the phenolic constituents of olive fruit and leaves have proven to be of significant importance both pharmacologically and in healthy diets. The plant is used widely in Tunisian traditional medicine. Oleuropein, demethyloleuropein, ligstroside, oleoside and verbascoside represent the predominant phenolic compounds isolated from the fruits, with oleuropein being generally the most abundant in all cultivars (Soler-Rivas et al. 2000). Excellent antioxidant properties were exhibited by oleuropein by inhibiting copper sulphate-induced oxidation of low-density lipoproteins (LDL), scavenging nitric oxide and affecting an increase in inducible nitric oxide synthase (iNOS) expression in the cells (Visioli et al. 2002).

From North Africa, *Alhagi maurorum* is a richly branched intricate shrublet which commonly grows in the Nile region including the Delta valley, Libyan Desert and the Red Sea coastal regions. Chemical investigation of *A. maurorum* revealed the presence of several flavonoids including tamarixetin 3-*O*-dirhamnoside and quercetin 3-*O*-rhamnoside and isorhamnetin. *A. maurorum* is used in folk medicine as a remedy for rheumatic pain, bilharzia, liver disorders, urinary tract infections and gastrointestinal disorders (Al-Jaber et al. 2011). Studies have shown that *A. maurorum* has peripheral and central antinociceptive activity at a dose of 400 mg/kg (Al-Jaber et al. 2011). Antioxidant activity has also been reported (Al-Jaber et al. 2011).

Among a tremendous list of flavonoids with excellent antioxidant properties reported from African medicinal plants, a significant number of these compounds have displayed antioxidant activities multiple-fold higher than the synthetic

Fig. 3.2 *Moringa oleifera* is successfully indigenised in many parts of Africa. (**a**) A private *Moringa* farm in Tooseng, Limpopo province of South Africa. (**b**) *Moringa* flowers. (**c**) *Moringa* seed pods about to mature

standard compounds. Of notable mention among this endless list, the prenylated flavonoids 6,8-diprenyleriodictyol and dorsmanins C and F, isolated from a Cameroonian medicinal plant *Dorstenia mannii*, were found to be have excellent DPPH radical scavenging activity when tested in a concentration range of 1–100 μM, displaying higher potency than butylated hydroxytoluene (BHT), a common antioxidant food additive. The compounds were also found to inhibit Cu^{2+}-mediated oxidation of human LDL in a dose-dependent manner (Dufall et al. 2003).

Compounds such as isoflavonoid burttinols A ($IC_{50,}$ 9.8 μM), B ($IC_{50,}$ 75.6 μM) and C ($IC_{50,}$ 10.6 μM), eryvarin H ($IC_{50,}$ 62 μM) and abyssinone V ($IC_{50,}$ 31.3 μM) identified from *Erythrina burttii* plants have also shown excellent DPPH radical scavenging properties (Yenesew et al. 2012).

The phenylpropanoid glycoside verbascoside, a compound isolated from, among other medicinal plant species, *O. europaea*, *N. laevis* (Table 3.2), *Halleria lucida* and *Buddleja davidii* displayed highly promising DPPH radical scavenging activity, with IC_{50} values of 7.18 μg/ml (Frum et al. 2007). Furthermore the anti-inflammatory activity of verbascoside has been confirmed by an in vitro test performed on cell cultures of primary human keratinocytes (Korkina et al. 2007), in which verbascoside was able to significantly reduce, in a dose-dependent manner, the release of pro-inflammatory chemokines. The study also demonstrated that verbascoside (Fig. 3.3) promotes skin repair and ameliorates skin inflammation due to its ROS-scavenging, antioxidant, iron-chelating and glutathione-S-transferase (GST) activity-inducing properties (Speranza et al. 2010; Kostyuk et al. 2011). An in vivo study, conducted on inflammation of the intestinal mucosa, demonstrated that verbascoside is able to inhibit the activation of pro-inflammatory proteins and consequently the enzymatic activity of matrix metalloproteinases, the latter of which is involved in skin ageing. The findings by Vertuani and colleagues suggested that verbascoside functions as an intracellular radical scavenger and thus reduces the microscopic and macroscopic signs of colitis rats, and thus administration of the compound may be beneficial for the treatment of inflammatory bowel disease (Vertuani et al. 2011).

The coumarin scopoletin (7-hydroxy-6-methoxychromen-2-one) (Fig. 3.3) is reported to possess excellent DPPH radical scavenging activity ($IC_{50,}$ 1.24 μg/ml) and inhibition potency comparably equal to that of the reference compound ascorbic acid ($IC_{50,}$ 1.22 μg/ml) under similar experimental conditions (Nazemiyeh et al. 2010). Xanthotoxol (Fig. 3.3), another coumarin, showed good 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)) radical scavenging activity ($IC_{50,}$ 10.3 μM) (Xiao et al. 2010).

Apart from the largely dominant polyphenols as antioxidants, some alkaloids with antioxidant properties have been identified from African medicinal plants. Of the alkaloid that exhibits significant antioxidants which include among others a panel of erythrinaline-type ((1)-erysodine, (1)-11α-hydroxyerysodine, (1)-erysotrine N-oxide, (1)-erythristemine), aristolactam alkaloids piperumbellactams A and B isolated from *Piper umbellatum* demonstrated an impressive DPPH radical scavenging activity, with IC_{50} values of 17.4 μM and 8.1 μM, respectively (Tabopda et al. 2008). From these findings, the authors postulated that the effectiveness of piperumbellactam B could be due to the presence of an ortho-dihydroxy group, which, by donating hydrogen radicals, gives greater stability to their radical forms. 1,3-dimethoxy-*N*-methylacridone has also been shown to inhibit the heme-mediated protein oxidation ($IC_{50,}$ 42 μM), while lycorine (Fig. 3.3) inhibits LPS-induced nitric oxide production ($IC_{50,}$ 1.2 μM) and LPS-induced TNF-α production ($IC_{50,}$ 0.9 μM) in the mouse macrophage cell line RAW264 (Pal et al. 2011; Yamazaki and Kawano 2011).

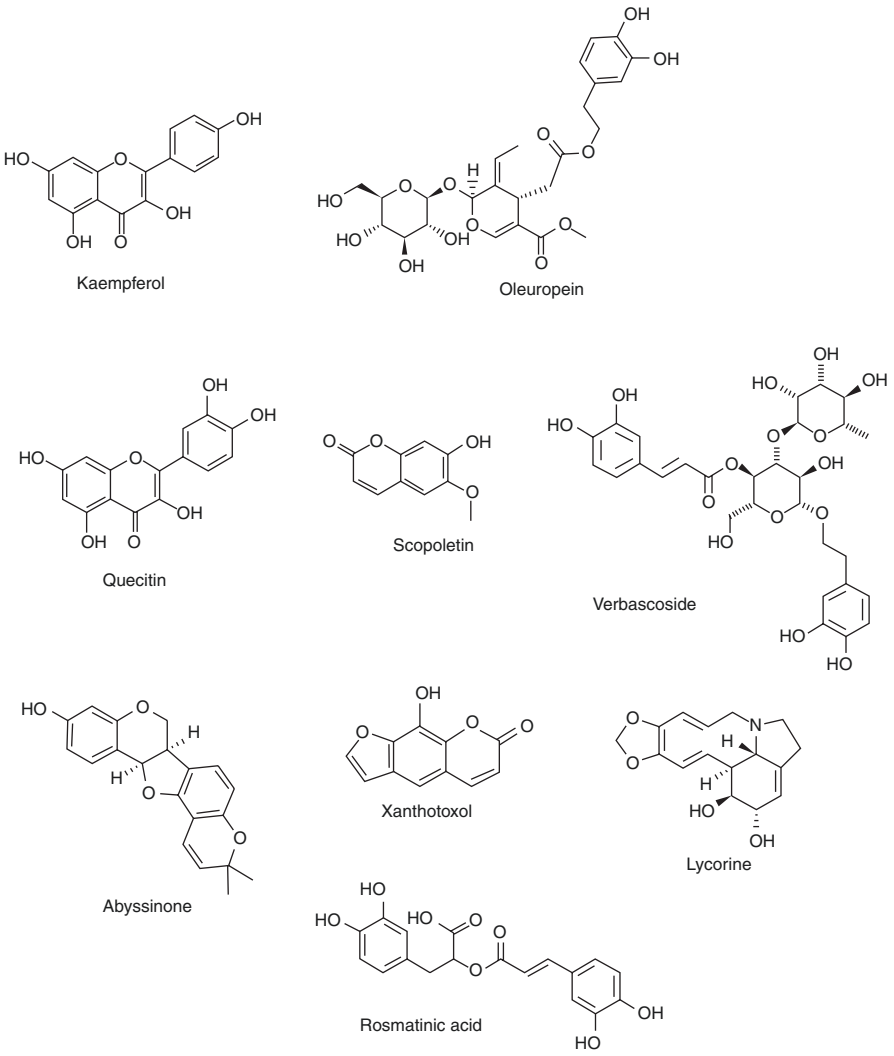

Fig. 3.3 Structural presentation of some potent compounds isolated from African medicinal plants with antioxidant activity

3.5 Conclusions

The health benefits of fruits, vegetables and medicinal plants are largely due to the antioxidant compounds such as vitamins and carotenes complemented with a large pool of phytochemicals, with varying degrees of antioxidant properties. Numerous medicinal compounds, some exhibiting significantly higher antioxidant properties than the commercially available synthetic standards, have been isolated from African medicinal plants. Owing to the rich and diverse flora of the African

continent, resident to different climatic regions, these groups of compounds span across a range of plant families and genera, with phenolics and alkaloids being some of the major classes of these compounds. Although significant progress has to date been made in identifying and isolating medicinal plant compounds, a number of African medicinal plants and foods have not yet received much attention as sources of antioxidants due to limited popularity and/or lack of commercial applications. However, the wealth of literature information on African medicinal plants indicates that these underexploited resources can potentially offer numerous health benefits in alleviating some diseases particularly those caused by oxidative stress. To realise the potential, of such sources of antioxidants, a more thoroughly focused evaluation of these plant resources, with an objective to develop complete compositional databases, precise antioxidant intake data and chemical characterisation and quantification, is thus essential.

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Chapter 4
Plant-Based Diets for Health Maintenance and Disease Prevention: Why and How?

Kaïs Hussain Al-Gubory

Abstract Organs and tissues of biological systems must control the production of reactive oxygen species (ROS) across the human life span. ROS are produced continuously as by-products of normal cell metabolism and also under stress conditions, such as malnutrition, undernutrition, environmental pollutants and unhealthy lifestyle behaviours. A fine balance between the levels of cellular ROS and enzymatic and non-enzymatic antioxidants is crucial for cell redox homeostasis and organ structural integrity and function. Disturbance of this balance causes oxidative damages of cellular macromolecules, induces mitochondrial defect and ultimately leads to organ dysfunction and increases the risk of development and progression of noncommunicable diseases (NCDs). Bioactive antioxidant compounds in plant foods can protect biological organs and tissues against ROS-induced oxidative stress, thereby promoting health and preventing or delaying the onset of NCDs. There are at least two possible preventive strategies for health maintenance and disease prevention: avoiding exposure to ROS-generating environmental factors and boosting cellular antioxidant defence capacity. Therefore, both regular intake of plant-based diets rich in antioxidants and healthy lifestyle behaviours can potentially prevent ROS-induced oxidative stress and associated NCDs. Considering the biodiversity and availability of plants worldwide and their beneficial health impacts, this chapter will review why and how plant-based diets and their bioactive antioxidant compounds, in addition to healthy lifestyle behaviours, can provide support for health maintenance and disease prevention.

Keywords Plant-based diets • Reactive oxygen species • Oxidative stress • Health maintenance • Disease prevention

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4.1 Introduction

The reactive oxygen species (ROS) are continuously generated in all living organisms as a result of normal cellular metabolism. Organs and tissues of biological systems have elaborated complex and interrelated antioxidant systems to keep in check the production of ROS under physiological conditions and also under conditions of stress, such as undernutrition, malnutrition, exposure to environmental pollutants and unhealthy lifestyle behaviours. Cellular (endogenous) and dietary (exogenous) antioxidants are components of interrelated systems that interact with each other to control ROS production and to ensure adequate defences against ROS-induced oxidative stress (Machlin and Bendich 1987). A fine balance between the levels of cellular ROS and endogenous and exogenous antioxidants is crucial for cell redox homeostasis, development and survival and organ structural integrity and functions. Disturbance of this balance causes oxidative damages of cellular macromolecules, including DNA, proteins and lipids (Valko et al. 2007), induces mitochondrial defect and ultimately leads to organ dysfunction and increases the risk of development and progression of noncommunicable diseases (NCDs) (Hernández-Aguilera et al. 2013; Ogura and Shimosawa 2014; Pagano et al. 2014) (Fig. 4.1).

Regular intake of plant-based diets rich in antioxidants and healthy lifestyle behaviours have the potential to prevent ROS-induced oxidative stress and associated NCDs. Bioactive antioxidant compounds in plant foods such as whole grains, fruit and vegetables (Zieli et al. 2000; Kaur and Kapoor 2001; Adom and Liu 2002; Adom et al. 2003; Liu 2003; Slavin 2003; Van Dokkum et al. 2008; Okarter and Liu 2010; Slavin and Lloyd 2012; Benisi-Kohansal et al. 2016; Rodriguez-Casado 2016; McRae 2017) and plant-derived beverages like wines (Actis-Goretta et al. 2002), tea (Siddiqui et al. 2004; Khan and Mukhtar 2007; Ruxton 2008; Afzal et al. 2015) and fruit or vegetable juices (Potter et al. 2011; Yuan et al. 2011; Foroudi et al. 2014; Tonin et al. 2015; Ekhlasi et al. 2016) can maintain an adequate organ and tissue antioxidant status, provide protection from oxidative stress and promote human health. Bioactive antioxidant compounds in plant foods and medicinal plants can also promote health and prevent or delay the onset of NCDs. Considering the biodiversity and availability of plants worldwide and their beneficial health impacts, this chapter reviews why and how plant-based diets and their bioactive antioxidant compounds, in addition to healthy lifestyle behaviours, can potentially support health maintenance and prevent disease. The health benefits of changes in diet habits and lifestyle behaviours are also highlighted.

4.2 Reactive Oxygen Species, Oxidative Stress and Noncommunicable Diseases

ROS include free radicals such as superoxide radical ($^{\bullet}O_2^-$), singlet oxygen ($^1O_2$), nitric oxide ($NO^{\bullet}$) and hydroxyl radical ($^{\bullet}OH$), peroxynitrite ($ONOO^-$) and nonradical species such as hydrogen peroxide (H_2O_2). ROS are produced primarily by the

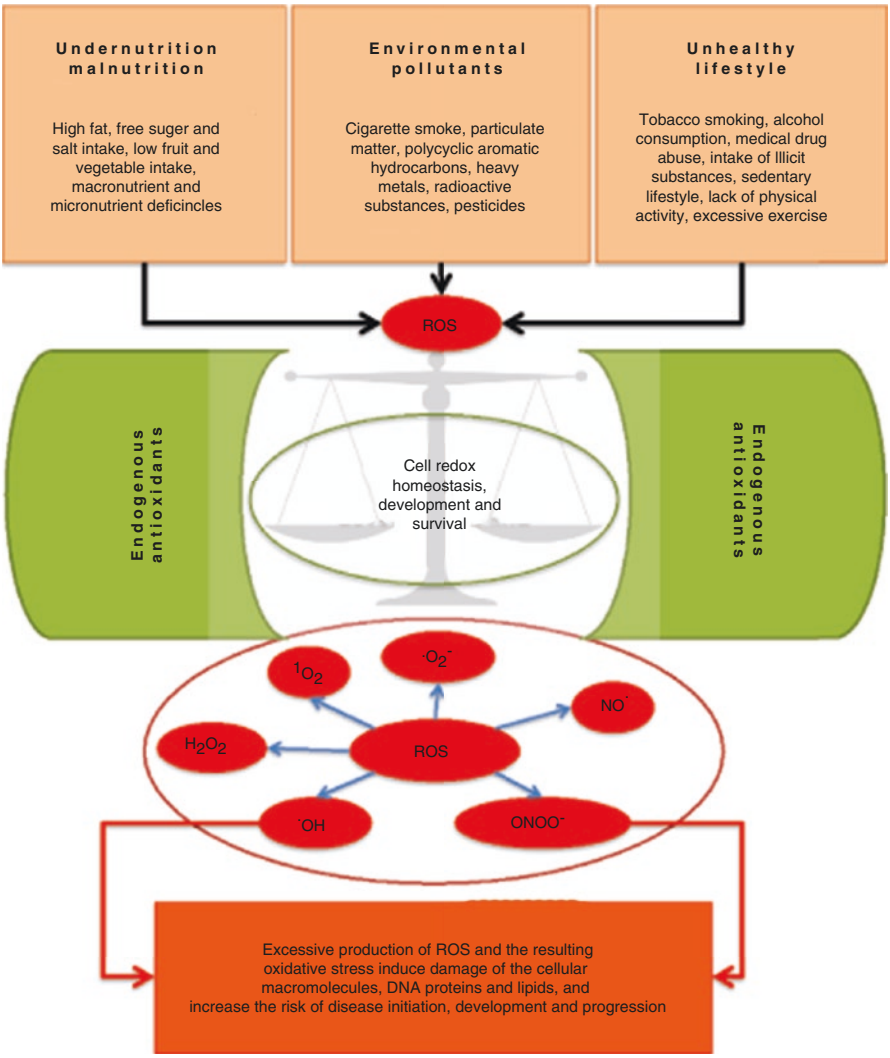

Fig. 4.1 A fine balance between the levels of cellular reactive oxygen species (ROS) and endogenous and exogenous antioxidants is crucial for cell redox homeostasis, development and survival and organ structural integrity and functions. Disturbance of this balance can cause oxidative damages of cellular macromolecules, including DNA, proteins and lipids, inducing mitochondrial defects, and can ultimately lead to organ dysfunction and increase the risk of development and progression of noncommunicable diseases. $^\cdot O_2^-$, superoxide radical; $^1O_2$, singlet oxygen; H_2O_2, hydrogen peroxide; NO$^\cdot$, nitric oxide; ONOO$^-$ peroxynitrite; $^\cdot$OH, hydroxyl radical

mitochondria as by-products of aerobic respiration and metabolism (Packer et al. 1996; Giulivi et al. 1998). Tightly regulated enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase isoforms (Nox) and NO synthases (NOS) also generate cellular ROS. The conversion of $^\cdot O_2^-$, H_2O_2 and NO$^\cdot$ within the

mitochondria to the highly reactive $^{\bullet}OH$ and $ONOO^{-}$ leads to oxidative damage of all major cellular constituents (Beckman et al. 1990; Packer and Murphy 1995; Packer et al. 1996).

ROS are cellular messengers (Khan 1995) and play important roles in numerous biological processes when they are produced at the physiological levels. The control of ROS levels by antioxidants is one of the central elements in cell signalling (Finkel 1998), gene expression, maintenance of redox homeostasis and signal transduction pathways involved in cell development and survival (Allen and Balin 1989; Allen and Venkatraj 1992; Allen and Tresini 2000). ROS are small diffusible molecules, and their release from mitochondria acts as second messengers to activate ROS-induced ROS release from neighbouring mitochondria (Zorov et al. 2006). Overproduction of $^{\bullet}OH$ and $ONOO^{-}$ as well as a decrease in cellular antioxidant capacity induces lipid peroxidation and can thereby damage cell membranes and alter their fluidity and membrane-bound protein synthesis (Ježek and Hlavatá 2005).

Oxidative stress is a state of imbalance between cellular production and removal of ROS. Under conditions where loss, inactivation and/or depletion of antioxidant defences occurs, disturbance of the cellular redox environment leads to diverse health disorders and complications. ROS interact with DNA causing epigenetic alterations in DNA methylation patterns that can affect gene expression and regulation (Cerda and Weitzman 1997). Replacement of guanine with the oxygen radical adduct, 8-hydroxyguanine, alters methylation of adjacent cytosine, suggesting a role for DNA oxidative damage in the formation of aberrant DNA methylation patterns during carcinogenesis (Cerda and Weitzman 1997). $NO^{\bullet}$ produced from the amino acid L-arginine by the vascular endothelium has a wide range of physiological actions that maintain vascular homeostasis, including regulation of blood pressure (Rees et al. 1989), microvascular tone (Persson et al. 1990), coronary vascular tone (Smith et al. 1992) and prevention of platelet aggregation (Gries et al. 1998).

The biological effects of $NO^{\bullet}$ in biological tissues depend on the balance between $^{\bullet}O_2^{-}$ and $NO^{\bullet}$ levels. Indeed, the reaction between $^{\bullet}O_2^{-}$ and $NO^{\bullet}$ leads to the formation of $ONOO^{-}$ and consequently reduces $NO^{\bullet}$ bioavailability. Excessive production of $^{\bullet}O_2^{-}$ plays a key role in the pathogenesis of atherosclerosis, at least in part by reducing the bioavailability $NO^{\bullet}$ (Violi et al. 2009). Nox-induced $^{\bullet}O_2^{-}$ generation plays an important role in type 2 diabetic nephropathy (Sedeek et al. 2010) and diabetes mellitus-accelerated atherosclerosis (Gray et al. 2013). Hence increased formation of $^{\bullet}O_2^{-}$ by Nox1 and Nox2 isoforms promotes endothelial dysfunction, atherosclerosis and hypertension (Gray and Jandeleit-Dahm 2015).

4.3 Endogenous and Exogenous Antioxidants

Tissues and organs of the body have highly complex and integrated endogenous enzymatic and non-enzymatic antioxidant systems, which function to maintain redox balance by regulating the generation and elimination of ROS. Copper (Cu) and zinc (Zn) superoxide dismutase (Cu,Zn-SOD or SOD1), manganese (Mn)

superoxide dismutase (Mn-SOD or SOD2), selenium (Se) glutathione peroxidase (Se-GPX or GPX), glutathione reductase (GR) and catalase (CAT) are localized in different cellular compartments, but they operate in co-ordinated manner to control ROS levels (Michiels et al. 1994). The SOD family is a ubiquitously distributed group of metalloenzymes. SOD1 is mostly located in the cytoplasm (McCord et al. 1971), whereas SOD2 is located in the mitochondria (Weisiger and Fridovich 1973). By catalysing the conversion of $^{\bullet}O_2^-$ into H_2O_2, SOD1 and SOD2 play major roles in the first line of defence against cellular oxidative stress. The GPX enzyme is located within the mitochondrial matrix and the cytoplasm, while CAT is found primarily within peroxisomes (Chance et al. 1979), and both enzymes catalyse the conversion of H_2O_2 to water (Fig. 4.2). Although GPX shares the substrate H_2O_2 with CAT, it alone can react effectively with organic hydroperoxides (ROOH) among which are fatty acid ROOH and nucleotide- or steroid-derived ROOH (Chance et al. 1979). The GR enzyme maintains adequate levels of reduced glutathione, which is necessary for the catalytic activity of GPX (Hayes and McLellan 1999). Glucose-6-phosphate dehydrogenase (G6PD), isocitrate dehydrogenases (ICDH), reduced/oxidized glutathione (GSH/GSSG) and reduced/oxidized NADPH/NADP also play important roles in the defence against oxidative stress. NADPH is necessary for the regeneration of GSH (Kirsch and De Groot 2001), and both G6PD and ICDH are crucial for cellular redox balance (Fig. 4.2) via maintenance of cytosolic NADPH (Tian et al. 1998; Jo et al. 2001).

The sulphur-containing amino acids, methionine, homocysteine, cysteine and taurine (2-aminoethanesulfonic acid) are also important components of the non-enzymatic antioxidant defence system (Atmaca 2004). They function as antioxidants through protection against $^{\bullet}O_2^-$ and its downstream ROS-induced oxidative stress. Homocysteine is the upstream amino acid for cysteine synthesis, and cysteine is the rate-limiting precursor for cellular taurine and GSH production (Fig. 4.2). Taurine acts as an antioxidant through protection against $^{\bullet}O_2^-$ and its downstream ROS-induced peroxidative damage (Alvarez and Storey 1983; Fellman and Roth 1985), and has implications in normal physiologic processes and pathologic conditions (Cañas and Valenzuela 1989), and preventive medicine (Kendler 1989). Taurine is the most abundant free amino acid and is found in many tissues and organs, especially in the muscle, kidney, brain and liver (Brosnan and Brosnan 2006).

Exogenous antioxidants, including plant polyphenolic compounds (Yu et al. 1997), beta-carotenes (Shih et al. 2008), vitamins E and C (Ryan et al. 2010) and trace elements (Klotz et al. 2003), are essential nutrients for enhancement and/or regulation of antioxidant enzymes in organs and tissues of biological systems and are used in health promotion (Pandey and Rizvi 2009; Santos et al. 2010; Kasote et al. 2015; Cicero and Colletti 2017). Trace elements such as Cu, Zn, Mn and Se act as cofactors and play crucial roles in the activities of SOD1, SOD2 and GPX. Undernutrition and malnutrition are major causes of deficiency of proteins, amino acids, trace elements and vitamins and consequently induce an imbalance between ROS and cellular enzymatic and non-enzymatic antioxidant systems. Under condition of poor nutrition, protein deficiencies also impair cellular antioxidant capacities because proteins provide the amino acids needed for the synthesis of antioxidant enzymes.

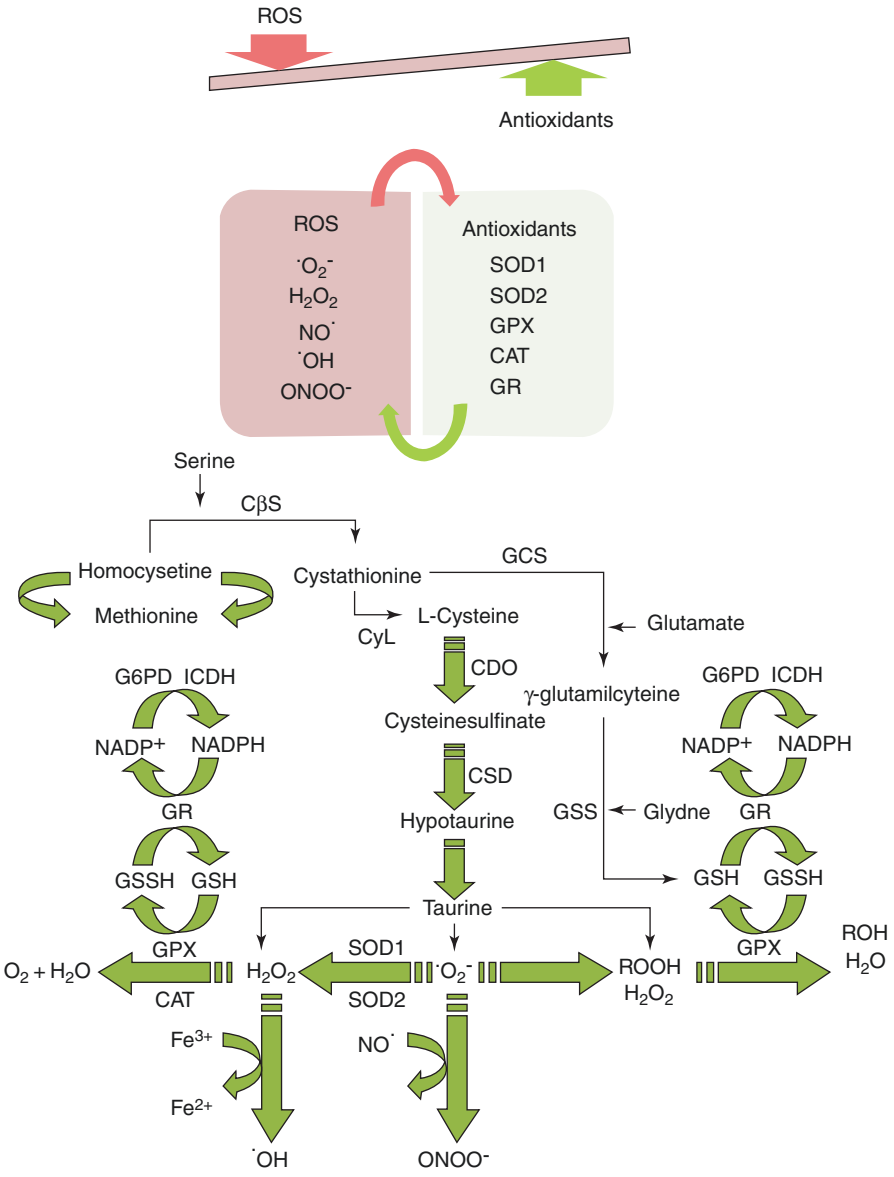

Fig. 4.2 Organs and tissues of biological systems have elaborated complex and interrelated enzymatic and non-enzymatic antioxidant pathways that function to keep in check the production of ROS. SOD1 or Cu,Zn-SOD, copper and zinc superoxide dismutase; SOD2 or Mn-SOD, manganese SOD; GPX, glutathione peroxidase; CAT, catalase; GR, glutathione reductase; ROS, reactive oxygen species; O_2, oxygen; $^{\cdot}O_2^-$, superoxide radical; H_2O, water; H_2O_2, hydrogen peroxide; $NO^{\cdot}$, nitric oxide; $ONOO^-$ peroxynitrite; $^{\cdot}OH$, hydroxyl radical; ROOH, hydroperoxides; ROH, alcohol; G6PD, glucose-6-phosphate dehydrogenase; ICDH, isocitrate dehydrogenases; NADPH/NADP, reduced/oxidized nicotinamide adenine dinucleotide phosphate; GSH/GSSG, reduced/oxidized glutathione; CBS, cystathionine b-synthase; GCS, gamma-glutamylcysteine synthetase; CγL, cystathionine gamma lyase; GSS, glutathione synthetase; CDO, cysteine dioxygenase; CSD, cysteine sulfinate decarboxylase

4.4 Phytonutrient Antioxidants, Biological Activities and Health-Beneficial Roles

Phytonutrients are bioactive compounds present in most foods of vegetable origin and are widely recommended for their health-promoting properties and disease prevention (Pandey and Rizvi 2009; Santos et al. 2010; Gupta and Prakash 2014). Phytonutrients have a wide range of biological activities such as antioxidative, antiproliferative, anti-inflammatory, antiobesogenic and antiangiogenesis (Upadhyay and Dixit 2015). A wide variety of medicinal plants (Cai et al. 2004) are also rich sources of antioxidants, which exhibit free radical-scavenging activity (Xu et al. 2017). It is important to note that vegetarians and vegans are at reduced risk of metabolic disorders and NCDs, including obesity, type 2 diabetes, hypertension, ischemic heart disease and certain types of cancer (Tonstad et al. 2009, 2013; Agrawal et al. 2014; Fraser et al. 2015; Melina et al. 2016). Phytonutrient antioxidants, including polyphenols, carotenoids, vitamins and trace elements, are essential components of fruits, vegetables and medicinal plants and integral parts of human diets (Kasote et al. 2015).

4.4.1 Polyphenols

Polyphenols are secondary plant metabolites and constitute the most abundant antioxidants diets (Bravo 1998). In addition to their ROS-scavenging action (Perron and Brumaghim 2009), polyphenols protect organs and tissues from ROS-induced oxidative damage by increasing the activity of antioxidant enzymes and thereby contribute to the beneficial health effects of dietary plants. Indeed, polyphenols stimulate endogenous antioxidant enzyme transcription through antioxidant responsive elements present in the promoter regions of many genes inducible by oxidative stress (Yu et al. 1997; Chen et al. 2000). This is supported by evidence from in vivo animal studies and also from in vitro studies of different cellular models.

Green tea, the most widely consumed beverage, is particularly rich in polyphenols known as catechins, which may constitute up to 30% of the dry leaf weight (Graham 1992). Feeding rats green tea leaves (2.5% in diet) for 27 days increases SOD and CAT activities, as well as GSH concentration in the liver (Lin et al. 1998). Tamarind (*Tamarindus indica L.*) fruit is a rich source of polyphenols (Bhadoriya et al. 2011). Healthy hamsters fed with dried powder from tamarind fruit pulp (500 mg/kg body weight) for 10 weeks exhibit higher levels of SOD, CAT and GPX activities in the liver compared to mice fed on a control diet (Lim et al. 2013). Methanol seed extract of tamarind seeds rich in polyphenolic compounds (572 ± 3.78 mg gallic acid equivalents (GAE/g dried plant material)) enhances activities of SOD, GPX and CAT in human liver HepG2 cells following H_2O_2-induced oxidative damage (Razali et al. 2015).

Pomegranate (*Punica granatum L.*) peel is a rich source of polyphenolic compounds with powerful antioxidant properties (Hasnaoui et al. 2014). Dried pomegranate peel contains 30% polyphenols and up to 8% and 5% of punicalagin and ellagic acid of the total polyphenols, respectively (Al-Gubory et al. 2016). Lipid peroxidation occurs in cell membranes when the hydrogen atom is abstracted from the unsaturated site in the fatty acid by $^{\bullet}OH$, resulting in the production of unstable lipid peroxide that decomposes to form malondialdehyde (MDA), the end product of polyunsaturated fatty acid peroxidation (Al-Gubory 2012). The small intestine of mice fed a diet supplemented with pomegranate peel powder (10% of diet) for 40 days ad libitum exhibits high levels of cytosolic SOD1 and GPX activities and low peroxidative stress as evidenced by reduced intestinal MDA content (Al-Gubory et al. 2016). Feeding rats with pomegranate peel (5% of diet) for 21 days counteracted the inhibitory effects of barium chloride (67 ppm) on kidney SOD, CAT and GPX activities and GSH levels (Elwej et al. 2016).

Polyphenol-rich extracts from the medicinal plants *Antirhea borbonica*, *Doratoxylon apetalum* and *Gouania mauritiana* protect 3T3-L1 preadipocytes against the anti-proliferative effect of H_2O_2 and also from adipose tissue inflammation induced by tumour necrosis factor alpha (TNFα) or lipopolysaccharide (LPS) endotoxin (Marimoutou et al. 2015). This protective action is associated with an increase in SOD gene expression and a decrease in mRNA levels of nuclear factor-κB (NF-κB) pro-inflammatory transcription factor. Polyphenols from *Antirhea borbonica* reverse the LPS-mediated expression of genes encoding ROS-producing enzymes in 3T3-L1 preadipocytes while also increasing the mRNA levels encoding mitochondrial SOD2 and CAT genes (Le Sage et al. 2017).

4.4.2 Carotenoids

Carotenoids are lipid-soluble phytoprotective pigments produced by most fruit and vegetables, but are not synthesized in animal cells (Bartley and Scolnik 1995). They have reactive conjugated double bonds in their structures and hence act as free radical scavengers (Rousseau et al. 1992). Carotenoids can play an important role in disease prevention by virtue of their antioxidant properties (Rousseau et al. 1992; Tee 1995; Edge et al. 1997). Although most carotenoids are composed of 40 branched carbon units bonded together, they have different structures and antioxidant activities. Of note is that lycopene quenches $^1O_2$ more than twice as effectively as β-carotene and that canthaxanthin and astaxanthin are more effective than β-carotene in chemoprevention (Rousseau et al. 1992). Lycopene and β-carotene (1–3 μmol) protect against DNA damage induced in human colon adenocarcinoma (HT29) cells by inhibiting xanthine/xanthine oxidase at relatively low concentrations (Lowe et al. 1999). Pretreatment of cultured human lymphocytes with astaxanthin (2 μM), a microalgae carotenoid and naturally present in some seafood, prevents oxidative stress induced by fatty

acids (0.3 mM), at least in part through reduction of ROS production (Campoio et al. 2011). The carotenoids, canthaxanthin, astaxanthin and β-carotene exhibit varying degrees of antioxidant activities as evidenced by their H_2O_2-scavenging effect in undifferentiated rat pheochromocytoma (PC12) cells (Chang et al. 2013). Feeding β-carotene and canthaxanthin for 6 weeks to rats placed on a high-fat diet (150 g/kg) or a high-cholesterol diet (10 g/kg) suppresses cholesterol-induced hepatic oxidative stress via enhancement of liver SOD, GPX, CAT and GR activities (Shih et al. 2008). Feeding β-carotene increases SOD, GPX, and CAT activities, whereas canthaxanthin feeding only increases GPX and CAT activities in erythrocytes (Shih et al. 2008). Epidemiological studies and clinical trials suggest that carotenoid supplementation provides beneficial effects in humans and may reduce the risk of ROS-mediated chronic disorders (Fiedor and Burda 2014; Cicero and Colletti 2017).

4.4.3 Vitamins

Vitamins including the water-soluble ascorbic acid (vitamin C) and the fat-soluble family of tocopherols (vitamin E) are well-known ROS-scavenging dietary antioxidants (Seifried et al. 2007). Tocopherols intercept lipid peroxyl radicals ($LOO^{\bullet}$) and terminate lipid peroxidation chain reactions in cell membranes. Ascorbic acid cannot scavenge lipophilic radicals within the lipid compartment, but it acts as a synergist with tocopherol for the reduction of $LOO^{\bullet}$ within the lipid compartment by reacting with tocopheroxyl radical and regenerating active tocopherol (Niki 1991). Ascorbic acid inhibits the intracellular generation of ROS in human myeloid HL-60 cells (Guaiquil et al. 2001). The resistance of the GSH-depleted myeloid HL-60 cells to H_2O_2-induced oxidative stress appears to be a consequence of intracellular consumption of ascorbic acid and generation of dehydroascorbic acid, an oxidized form of ascorbic acid (Guaiquil et al. 2001). Treatment of human 293T kidney cells with 5 mM H_2O_2 and 100 μM Cu^{2+} increases mutation frequency by eightfold, whereas pretreatment of these cells with dehydroascorbic acid (500 μM) for 60 min reduces the mutation frequency induced by H_2O_2/Cu^{2+} (Lutsenko et al. 2002). Importantly, dehydroascorbic acid prevents H_2O_2-induced cell death in primary astrocytes by increasing GPX and GR activities and GSH levels (Kim et al. 2005). In patients with systemic lupus erythematosus, an autoimmune disease also known as lupus, combined administration of vitamins C (500 mg) and E (800 IU) daily for 12 weeks decreases plasma MDA concentration (Tam et al. 2005). Alpha (α)-tocopherol, a predominant vitamin E isomer found in the body, is an efficient scavenger of lipid peroxyl radicals (Wang and Quinn 1999). Supplementation with α-tocopherol acetate (2,000 units/kg diet) in TGFα/c-myc transgenic mice starting from 3 weeks old until 10 weeks of age reduces ROS formation and protects against initiation and progression of hepatocarcinogenesis (Factor et al. 2000). This supplementation corresponds to ≈10 IU of vitamin E/day/mouse based on food consumption (5 g) per day (Factor et al. 2000).

Daily supplementation of vitamin C (1 g) and α-tocopherol acetate (1000 IU) for 8 weeks reduces production of ROS, such as $^{\bullet}O_2^-$ and $NO^{\bullet}$, and thiobarbituric acid reactive substances (TBARS, a marker of lipid peroxidation), in the retina of pigs pretreated with a hypercholesterolemic diet (24.5% animal lard, 4% cholesterol) that induces oxidative stress and ultrastructural alterations in retinal pigment epithelium (Fernandez-Robredo et al. 2005).

4.4.4 Trace Elements

The trace elements Se, Cu, Zn and Mn are present in minute quantities in the body and act as essential cofactors to the structure and biological activities of the antioxidant enzymes Cu,Zn-SOD, Mn-SOD and Se-GPX (Klotz et al. 2003). Substantial evidence from animal studies indicates that oxidative stress, resulting from a deficiency in trace elements, modulates activities of trace element-dependent antioxidant enzymes. Under Cu depletion condition (0.8 mg Cu/kg diet) for 6 weeks, Cu,Zn-SOD activity decreases in rat liver, erythrocyte and heart, whereas Cu repletion increases Cu,Zn-SOD activity in the liver and heart (Paynter et al. 1979). In the liver of Mn-sufficient rats, Mn-SOD activity increases over sixfold from birth to 60 days of age, whereas in the liver of Mn-deficient rats, activity of this enzyme increases only threefold during the same time period (Zidenberg-Cherr et al. 1983). Moreover, a significant increase in mitochondrial lipid peroxidation was observed at 60 days of age in the liver of Mn-deficient rats, suggesting that mitochondrial membrane damage may be due to depressed Mn-SOD activity (Zidenberg-Cherr et al. 1983). The liver and heart of rats fed a Cu-deficient diet (0.6 mg Cu/kg diet) exhibit reduced Cu,Zn-SOD activity compared to the activity in rats fed a control diet (6 mg Cu/kg diet) (L'Abbé and Fischer 1984). The lungs of mice, rendered Mn deficient (1 μg Mn/g diet) and Cu deficient (0.2 μg Cu/g diet) and then exposed to oxidant stress (7 days exposure to 1.2 ppm ozone), exhibit low activities of Mn-SOD and Cu,Zn-SOD (Dubick et al. 1988). Myocardial Se-GPX activity is 24% lower in the Se-deficient (<0.01 mg Se/kg diet) rats than in control rats supplemented with 0.5 mg Se/l in drinking water (Ji et al. 1992). Erythrocyte Cu,Zn-SOD activity decreases by 46% in Cu-deficient rats (<1 mg Cu/kg diet) and increases to control levels after 1 day of Cu supplementation (CuS; 5 mg/l) in the drinking water (Panemangalore and Bebe 1996). Turnlund et al. reported that the minimum dietary Cu requirement of young men is between 0.4 and 0.8 mg/day and that intake of <0.4 mg/day decreases the activity of erythrocyte SOD (Turnlund et al. 1997). Malnutrition in children is associated with a deficiency in serum Zn (2.59 μg/ml in the malnutrition group vs. 3.92 μg/ml in the control group) and Cu (0.74 μg/ml in the malnutrition group vs. 1.19 μg/ml in the control group) that leads to a decrease in SOD activity (21.13 U/mg protein in the malnutrition group vs. 26.02 U/min per mg protein in the control group) (Thakur et al. 2004).

4.5 Plant-Based Diets for Health Promotion and Disease Prevention

Plant polyphenols have been extensively studied for their beneficial health effects in humans, with evidence that populations consuming diets rich in polyphenols are less susceptible to age-related pathologies. This association is discussed in greater depth in other chapters of this book. It is now generally accepted that the antioxidant activity of polyphenols explains the human health benefits of diets rich in fruit and vegetables (Pérez-Jiménez et al. 2010). Many polyphenols present in fruit and vegetables, including herbal plants, cereal grains, nuts, seeds and spices, as well as plant-derived beverages, promote health and prevent diseases (Carlsen et al. 2010). Observational studies have long identified that certain populations of Mediterranean countries that ingest relatively higher levels of polyphenols tend to be better protected from obesity, type 2 diabetes and cardiovascular diseases (Martínez-González et al. 2015).

4.5.1 Plant-Based Diets for Healthy Foetal and Neonatal Life

Poor maternal antioxidant status and oxidative stress are associated with adverse prenatal development outcomes (Luo et al. 2006), while maternal antioxidant redox status positively impacts birth weight (Osorio et al. 2011). Micronutrient deficiencies lead to in utero developmental impairment and pregnancy complications (Keen et al. 2003; Christian and Stewart 2010). Maternal malnutrition is the main factor that contributes to an unhealthy pregnancy and increases the susceptibility of offspring to development disorders and complications via ROS generation and oxidative cell damage (Gupta et al. 2004; Luo et al. 2006; Franco et al. 2007; Lakshmy 2013).

Maternal gestational diabetes disturbs antioxidant/pro-oxidant balance and induces oxidative stress in the newborn. Erythrocyte MDA levels were elevated in babies born to mothers with gestational diabetes (Kamath et al. 1998). Cord blood collected from newborns of mothers with diabetes has lower total antioxidant status and higher level of TBARS (Bis-Głuchowska et al. 2001). Cord blood and placental homogenates collected after delivery from diabetic women have higher MDA levels (Kinalski et al. 2001). SOD activity and markers of oxidative stress (8-isoprostane and protein carbonyl) are elevated in placentas obtained from women with gestational diabetes mellitus (Coughlan et al. 2004). Mothers with gestational diabetes and their macrosomic babies exhibit low SOD activity and high TBARS levels in their serum (Grissa et al. 2007). Macrosomia is a predominant adverse outcome in cases of maternal gestational diabetes (Mitanchez 2010). Maternal preeclampsia is a serious condition of pregnancy and is a leading cause of maternal mortality and morbidity in developing countries (Berg et al. 2009). The onset of preeclampsia is characterized by defective placentation, hypoxia and oxidative stress (Hansson et al. 2015).

Preconception obesity, identified in women of reproductive age as a malnutrition-related preventable disorder (Guelinckx et al. 2008), is one of the risk factors of adverse prenatal development and birth outcomes and is also a major determinant of offspring health during childhood and adult life (O'Reilly and Reynolds 2013, Godfrey et al. 2017). Maternal obesity before conception and during the first trimester increases the risk of a number of pregnancy complications, including gestational diabetes mellitus, large for gestational age or macrosomia (foetal weight of ≥4.5 kg), gestational hypertension and preeclampsia (Leddy et al. 2008). Obese women and their offspring are at increased risk for diabetes, and daughters of obese women have a risk of obesity and heart disease (Leddy et al. 2008). Maternal obesity before and during pregnancy has severe adverse effects on prenatal development and pregnancy outcome and therefore requires preventive nutritional strategies to reduce the risk of foetal developmental disorders and maternal pathologies (Simmons 2011). In population-based studies in women, a dietary pattern characterized by high intakes of vegetables (Longo-Mbenza et al. 2008; Torjusen et al. 2014) and a Mediterranean-style dietary pattern (Schoenaker et al. 2015) decrease the risk of developing hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia. Rutgers Scarlet Lettuce (*Lactuca sativa L.*) contains high amounts of polyphenols (8.7 mg/g fresh weight gallic acid equivalents). Daily oral administration of Rutgers Scarlet Lettuce (100 or 300 mg/kg) for up to 8 days reduces hyperglycaemia and improves insulin sensitivity in high-fat diet-induced obese hyperglycaemic mice (Cheng et al. 2014).

The beneficial effect of diets rich in natural antioxidants on fertility, prenatal developmental and offspring health outcomes is an attractive idea of great interest for human reproductive medicine. Studies examining the consumption of plant products that are rich in antioxidants as preventive strategies of pregnancy-related disorders and complications are necessary to confirm its beneficial effects and its safety for the mother and the developing foetus. Whether or not regular and/or increased intake of phytonutrients and plant-derived diets can prevent preconception obesity and its associated prenatal complications has not been explored.

4.5.2 Plant-Based Diets for Prevention of Adult Diseases

A food frequency questionnaire study of 69,017 women aged 38–63 years without history of major chronic diseases reveals that a diet high in fruit, vegetables, whole grains, legumes, poultry and fish and low in refined grains, potatoes and red and processed meats may lower the risk of coronary heart disease (Fung et al. 2001). Plant-based dietary interventions (a vegetarian or vegan diet combined with nuts, soy and/or fibre) can lower plasma low-density lipoprotein and total cholesterol concentrations by up to 35% (Ferdowsian and Barnard 2009) suggesting that populations following vegetarian and vegan diets are at lower risk for ischaemic heart disease mortality. A systematic search of the literature published earlier than 2015

was conducted in Medline, PubMed, Scopus, Embase and Cochrane Library to identify studies on the relation between the consumption of whole grains and the risk of mortality (Benisi-Kohansal et al. 2016). This meta-analysis of prospective cohort studies reveals an inverse association between whole-grain intake and all-cause mortality, cardiovascular disease and total cancers. Another PubMed search from January 1, 1980, to May 31, 2016, was made to review the effectiveness of whole grains as therapeutic agents in type 2 diabetes, cardiovascular disease, cancer and obesity (McRae 2017). This review reports a statistically significant positive correlation between dietary whole-grain intake and reduction in the incidence of type 2 diabetes (relative risk "RR" = 0.68–0.80), cardiovascular disease (RR = 0.63–0.79) and colorectal, pancreatic and gastric cancers (RR = 0.57–0.94), as well as significant reductions in cardiovascular and cancer mortality (RR = 0.82 and 0.89, respectively). This meta-analysis suggests that a daily intake of two to three servings (~45 g) of whole grains may be beneficial in the prevention of NCDs (McRae 2017).

Dietary factors including sugars, sweeteners, total fat, monounsaturated fatty acids, total polyunsaturated fatty acids (PUFAs), omega-3 fatty acids and *n*-6 fatty acids are implicated in the pathogenesis of inflammatory bowel disease (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC) (Sakamoto et al. 2005; Ananthakrishnan et al. 2014; Owczarek et al. 2016); these gastrointestinal disorders are common in Europe and North America (Molodecky et al. 2012). The increase in IBD prevalence in industrialized countries is associated with unhealthy lifestyle behaviours, including intake of diets rich in sugar, saturated fats and red meats (Schaubeck and Haller 2015). High intake of saturated fats, monounsaturated fatty acids, total PUFAs, omega-3 fatty acids, omega-6 fatty acids, mono- and disaccharides and meat increases the risk of CD and UC, whereas diet rich in fibre and fruit decreases CD risk; likewise a diet with high amounts of vegetables decreases UC risk (Hou et al. 2011). Long-term intake of fibre from fruit is associated with lower risk of CD but not UC in women (Ananthakrishnan et al. 2013).

The human gut contains a complex microbial ecosystem (bacterial microbiota) that plays an important role in maintaining healthy intestinal functions, either directly or indirectly, by modifying food components or endogenously producing signalling molecules (Bäckhed 2012). Altered microbiota composition under condition of IBD and obesity (Bäckhed 2012) ultimately disturbs the interactions between the host and its microbiota (Berry and Reinisch 2013; Schaubeck and Haller 2015). The biological properties and health effects of dietary polyphenols are dependent on their bioavailability. This includes biotransformation of polyphenols by gut microbiota, modulation of gut microbiota by polyphenols and the effects of these two-way interactions on polyphenol bioavailability (Ozdal et al. 2016). Dietary polyphenols also contribute to the maintenance of intestinal health by preserving the gut microbial balance through the stimulation of the growth of beneficial bacteria (e.g. lactobacilli and bifidobacteria) and the inhibition of pathogenic bacteria (Dueñas et al. 2015).

4.5.3 Plant-Based Diets for Healthy Ageing

Ageing is characterized by a progressive decline in the efficiency of organ function and by the increased susceptibility to disease. Excess production and accumulation of ROS, which affect enzyme activity and membrane function, may overwhelm endogenous antioxidant defences leading to cellular functional impairment. Upregulation of endogenous antioxidant enzymes by dietary intervention to prevent age-related diseases is of importance for healthy ageing. Basic research and epidemiological studies suggest that dietary antioxidants, including plant polyphenols, protect against age-related pathologies. A variety of mechanisms have been proposed through which polyphenols reduce oxidative damage, including direct scavenging of ROS, inhibition of ROS production pathways and increase of the endogenous antioxidant pathways.

Dietary polyphenols present an opportunity to limit age-related declines in organ functions and consequently help to manage or prevent disease in older adults. Consumption of fruit and vegetables is an important part of a healthful diet for the elderly. Daily supplementation of two cups of frozen blueberries for 6 weeks counteracts age-related decline in functional mobility in older adults (Schrager et al. 2015). Sarcopenia is a geriatric syndrome characterized by a decrease of muscle mass, which can lead to a deterioration of the muscular strength and physical performance. Sarcopenic obesity, a new type of obesity, is characterized by age-related body fat gain, loss of muscle mass and poor muscle strength (Stenholm et al. 2008; Zamboni et al. 2008) that contribute to type 2 diabetes, dyslipidaemia and cardiovascular disease (Kim and Choi 2015). Frequent dietary intake of fruit and vegetables reduces the risk of sarcopenia in elderly men and women aged ≥65 years (Kim et al. 2015).

4.6 Safety and Nutritional Value of Plant-Based Organic Foods

As a consequence of the increasing importance of plant-based diets for health maintenance and disease prevention, there is great interest in the quality of fruit and vegetables. Consumers prefer natural plant foods with high nutritional value that promote good health. In addition, consumers are concerned about functional plant foods, mainly those without genetically modified organisms, which are fresh, natural and minimally processed. Food consumption and eating habits must have health and environmental impact. Water pollution, soil degradation, reduced biodiversity, the presence of chemical residues in food and their related health risks have serious implications for the sustainability of conventional agricultural systems (Reisch et al. 2013).

Conventional fruit and vegetables can be contaminated by a large number of pesticides as a result of increasing treatments of crops in both developed and developing countries (El-Saeid 2003; Katz and Winter 2009; Keikotlhaile et al. 2010; Probst et al. 2010; Winter 2012; Cervera et al. 2014; Wang et al. 2014). A recent

study reveals the presence of 147, 145, 141 and 131 pesticide residues in apple, potato, cabbage and spinach, respectively (Saito-Shida et al. 2016). Early-life exposure to organochlorine pesticides (POPs) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) adversely influences the development and maturation of the immune and respiratory systems (Lundqvist et al. 2006; Gascon et al. 2013) and increases the risk of immune-related diseases and lung dysfunction in offspring in later life (Cao et al. 2016). Considering the vulnerability of infants to environmental chemical exposure, the presence of detectable levels of POPs in infant foods is a major health concern (Jeong et al. 2014; Toms et al. 2016). Even some pesticides (mostly unauthorized for use in conventional agricultural systems) detected in fruit and vegetables below the maximum residue level are endocrine-disrupting chemical (Mutengwe et al. 2016). Importantly, meta-analyses based on 343 peer-reviewed publications indicate that organic crops have higher concentrations of polyphenolic antioxidants, such as phenolic acids, flavanones, stilbenes, flavones, flavonols and anthocyanins, and lower concentrations of cadmium and pesticide residues compared to the non-organic comparators across regions and production seasons (Barański et al. 2014).

Plant ROS production is a common factor or outcome of exposure to environmental stress conditions (Apel and Hirt 2004). Secondary metabolites play an important role in the adaptation of plants to environmental factors, and accumulation of such metabolites occurs in plants subjected to abiotic and biotic stresses (Ramakrishna and Ravishankar 2011; Suzuki et al. 2014). Under natural field conditions, plants respond to the aggressions of abiotic and biotic environmental stress factors by increasing their antioxidant capacity (Pérez-López et al. 2013; Sales et al. 2013; Rivero et al. 2014).

It can be concluded that natural field conditions can impact the quality of plants and plant-derived products. Levels of the polyphenols, epigallocatechin gallate (EGCG) and catechin gallates (CGs), in fresh tea shoots grown in Australia, are higher in the warmer months of April (120.52 mg/g and 163.75 mg/g, respectively) and May (128.63 mg/g and 183.83 mg/g, respectively) and lower during the cooler months of July (91.39 mg/g and 132.30 mg/g, respectively), suggesting that day length, sunlight and/or temperature influences tea polyphenol content (Yao et al. 2005). Likewise, polyphenol content (Romania et al. 2002; Oh et al. 2011) and antioxidant capacity (Travieso et al. 2016) are lower in leaves of lettuce cultivated under greenhouse conditions than those in leaves derived from lettuce grown on open field.

4.7 Conclusions

Plant antioxidants can protect biological organs and tissues from ROS-induced oxidative stress, thereby promoting health and preventing or delaying the onset of NCDs. However, in the present state of knowledge, it is inconceivable to imagine that supplementation with one or several antioxidants in combination could prevent ROS-induced cellular oxidative stress, since the onset and progression of diseases are complex and difficult to control under conditions of organ dysfunction. Phytonutrients, mainly

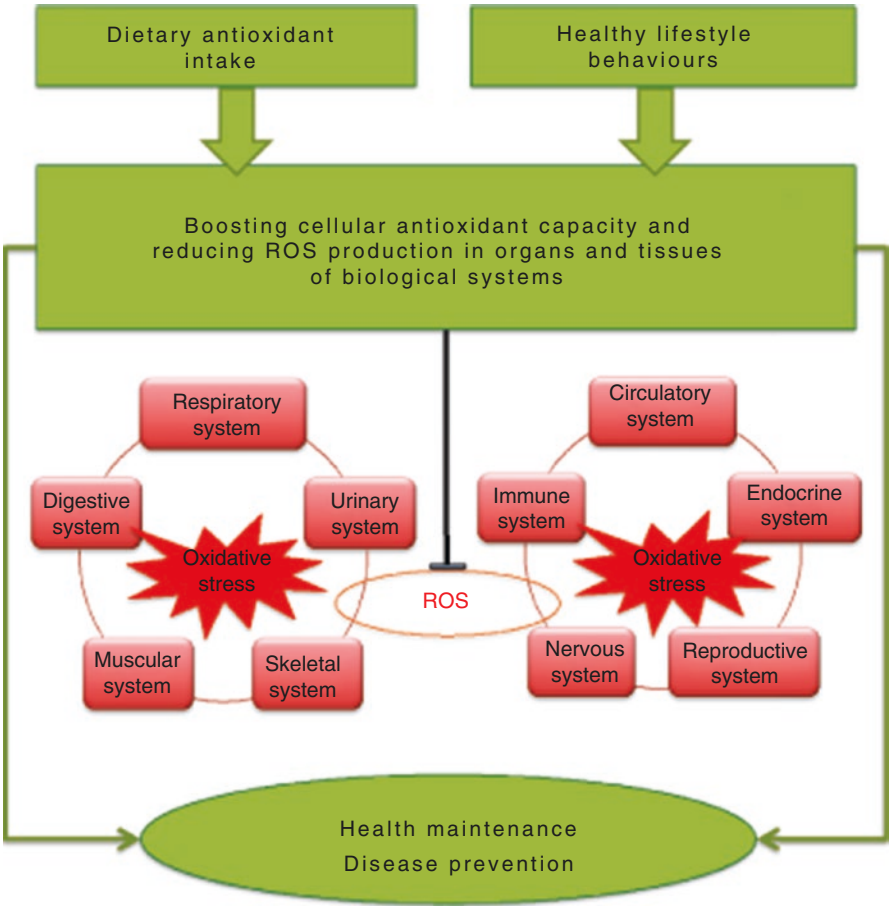

Fig. 4.3 Health maintenance and disease prevention require intake of dietary antioxidant and healthy lifestyle behaviours that can boost the cellular antioxidant capacity and reduce formation of reactive oxygen species (ROS) in organs and tissues of biological systems

antioxidant vitamins, trace elements, carotenoids and polyphenols, act synergistically to scavenge ROS within biological systems. A combination of different plant-based antioxidant compounds provides some protection of the body from oxidative damage.

Plant antioxidants can improve antioxidative status and quality of life. Bolstering endogenous enzymatic antioxidant defence pathways by plant-based diets and their bioactive antioxidant compounds may be an effective mean to protect organs and tissues of biological systems from toxicity of ROS (Newsome et al. 2014; Al-Gubory et al. 2016). There are at least two possible preventive strategies for health maintenance and disease prevention: avoiding exposure to ROS-generating environmental factors and boosting cellular antioxidant defence capacity (Fig. 4.3). Therefore, both regular intake of diets rich in antioxidants and healthy lifestyle behaviours are potentially valuable strategies to prevent ROS-induced oxidative stress and associated NCDs.

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Chapter 5
Antioxidants in Reproductive Health and Fertility

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Abstract Antioxidants play a vital role in reproductive health and fertility. The local cellular environment influences oocyte development, ovulation, successful fertilization, and maintenance of early pregnancy. An offset of reactive oxygen species (ROS)-induced cellular oxidative stress can adversely affect function of reproductive organs and fertility. ROS and antioxidants have emerged as ubiquitous participants in normal human reproductive processes. Imbalance in the oxidant/antioxidant relationship has been implicated in numerous reproductive disorders and complications, including endometriosis, polycystic ovarian syndrome, oocyte aging, dysmenorrhea and premenstrual syndrome, spontaneous abortion, and infertility. Studies have examined dietary antioxidant supplementation in hopes to prevent and treat reproductive pathologies. Dietary antioxidants, including isoflavones, antioxidant vitamins, and trace elements, which are cofactors of key antioxidant enzymes, have been targeted as nutritional antioxidants for prevention and/or

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treatment of unexplained infertility and recurrent spontaneous abortions. However, there is a vast knowledge deficit to fill, and targeted studies are needed to elucidate the role of ROS in normal reproductive physiology and fertility, as well as in treatment of reproductive pathologies. This chapter reviews the role played by antioxidants in female reproductive health and fertility and antioxidant interventions for the prevention and treatment of reproductive pathology in association with oxidative stress.

Keywords Oxidative stress • Reproductive disorders and complications • Antioxidants • Female reproductive health • Fertility

5.1 Introduction

Intracellular homeostasis is maintained at least in part by a fine balance between oxidants and antioxidants. Oxidative stress is a state associated with increased damage to cellular structure and function induced by oxygen-derived oxidants commonly known as reactive oxygen species (ROS), which are by-products of aerobic respiration and metabolism. ROS production is tightly controlled by interrelated antioxidant systems that modulate intracellular ROS concentration and set the redox status of the cell. The common ROS that have potential implications in mammalian reproduction and developmental biology are superoxide radicals ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), nitric oxide ($NO^{\bullet}$), and peroxynitrite anion ($ONOO^-$). ROS and antioxidants have emerged as ubiquitous participants in normal human reproductive processes, and an imbalance in the oxidant/antioxidant relationship has been implicated in numerous reproductive pathologies, including endometriosis, polycystic ovarian syndrome and infertility (Agarwal et al. 2005; Fujii et al. 2005), and prenatal development disorders and complications (Al-Gubory et al. 2010).

Maternal undernutrition or poor nutrition can have long-lasting effects on reproductive potential. Infertility due to ovulation disorders may be preventable by healthy diet and lifestyle behaviors (Chavarro et al. 2007). Substantial evidence suggests that low antioxidant status and oxidative stress may be associated with infertility of both known and idiopathic origin (Ruder et al. 2008). Rats subjected to caloric undernutrition (50% of a standard control diet) during pregnancy and lactation exhibited reduced primary and secondary follicle counts in their offspring (Bernal et al. 2010). These offspring were found to have increased ovarian protein carbonyl groups (biomarkers of oxidative stress), implying decreased antioxidant defenses and increased oxidative damage. It is speculated that oxidative stress accelerates reproductive maturity along with faster decline in ovarian reserve (Bernal et al. 2010). Deficiency in vitamin C, a well-known antioxidant, has been associated with increases in premature births and predisposing newborns to oxidative stress (Negi et al. 2012).

There is a wide array of plant antioxidants, which have a variety of functions but have in common the ability to neutralize ROS in order to maintain homeostasis and protect cells from undue oxidative damage. There is growing interest in the effects of increased consumption of dietary phytoestrogens on the spectra of reproductive processes, including the timing of menarche and menopause. Natural antioxidants have been targeted as nutritional therapies for recurrent spontaneous abortions and unexplained infertility (Sekhon et al. 2010), and some preliminary studies examining antioxidant supplementation in the periconception period have been encouraging (Cetin et al. 2010; Mistry and Williams 2011; Twigt et al. 2012; Al-Gubory 2013). However, there is a vast knowledge deficit to fill, and targeted studies are needed to elucidate the role of ROS in normal reproductive physiology and fertility, as well as in reproductive pathologies. In this chapter, we review the major dietary antioxidants implicated in female reproductive health and fertility. This chapter also reviews the role played by antioxidants in female reproductive health and fertility and antioxidant interventions for the prevention and treatment of reproductive pathologies in association with oxidative stress.

5.2 Dietary Antioxidants

Dietary antioxidants, including isoflavones, tocopherol (vitamin E), provitamin A carotenoids, and ascorbate (vitamin C), as well as dietary antioxidant cofactors, mainly copper, zinc, manganese, and selenium, have been targeted as nutritional antioxidants for prevention of recurrent spontaneous abortion and treatment of unexplained infertility, and some preliminary studies examining antioxidant supplementation during the periconception period have been encouraging (Ramakrishnan et al. 2012; Gernand et al. 2016).

5.2.1 Isoflavones

Phytoestrogens are nonsteroidal compounds that mimic the conformation of estradiol and are able to bind to estrogen receptors and interact with estrogen signaling pathways. They have a higher affinity for estrogen receptor-beta than for estrogen receptor-alpha (Cederroth et al. 2012). Soy products are the predominant source of dietary phytoestrogens in humans, the most studied subclass being the isoflavones (i.e., genistein, daidzein, and glycitein—other subclasses include flavonols, catechins, etc.). Whereas soy products had been rare in the Western diet with the exception of oils, more and more processed foods, including granola bars, cereals, hot dogs, sausages, and infant formula, now contain soy products as fortifiers and meat substitutes (Patisaul and Jefferson 2010; Cederroth et al. 2012). Dietary intake varies widely between individuals (e.g., the "typical" Asian diet results in isoflavone consumption as high as 50 mg/kg body weight/day, whereas 1–3 mg/day total is

more common in the Western diet) (Mortensen et al. 2009). As a point of comparison, infants fed exclusively soy formula consume approximately 6–9 mg/kg body weight/day in isoflavones (Cao et al. 2009), resulting in a mean plasma isoflavone concentration of 980 μg/L (compared to 9.4 μg/L in breast-milk-fed infants and 4.7 μg/L in cow's milk-based formula-fed infants) (Cederroth et al. 2012). Seasonal variation also influences the phytoestrogen content of foods (e.g., the total isoflavone content of raw soybeans can range from 18 to 562 mg/100 g) (Mortensen et al. 2009).

Phytoestrogens from soy protein products are able to alter the synthesis, secretion, metabolism, and transport of natural hormones in the body. As a result they can affect such processes as sexual development and puberty, gamete production, pregnancy, and lactation (Patisaul and Jefferson 2010; Cederroth et al. 2012; Kim and Park 2012). In fact, the endocrine-disrupting properties of soy isoflavones were first discovered when a group of ewes grazing in a red clover patch developed infertility, later attributed to a phytoestrogen compound in red clover called formononetin (Cederroth et al. 2012). Isoflavones regulate plasma sex hormone-binding globulin (sHBG) levels and aromatase and 5-α reductase and displace testosterone and 17-β estradiol from sHBG sites (Patisaul and Jefferson 2010; Cederroth et al. 2012). As powerful as soy compounds have the potential to be, the effects and impact of soy compounds on reproductive health are not yet clear, as the differences in nomenclature, formulations, dosages, routes, times, and durations of administration are not standardized across studies and are therefore difficult to compare.

5.2.2 Antioxidant Vitamins

Vitamin E, vitamin C, and the carotenoids are among the major dietary antioxidants (Rock et al. 1996; Johnson et al. 2003). Vitamin E is a group of eight fat-soluble compounds that functions as a direct antioxidant. The discovery of vitamin E in 1922 and its essential role in rat reproduction is credited to Evans and Bishop (1922). Since that time, countless studies have been undertaken to determine the specific function of vitamin E and to test the potential benefits of vitamin E supplementation, particularly in fertility, that to date have been inconclusive (Brigelius-Flohe and Galli 2010). ß-carotene, the main source of provitamin A, and vitamin C (ascorbic acid) are both important ROS scavenging antioxidants (Burton and Ingold 1984; Weber et al. 1996).

5.3 Antioxidant Enzymes and Cofactors

The essential nutrients copper, zinc, manganese, and selenium are important factors in maintaining health, reproduction, and fertility (Bedwal and Bahuguna 1994). They are cofactors for copper, zinc-superoxide dismutase (Cu, Zn-SOD, or SOD1),

manganese-SOD (Mn-SOD or SOD2), and selenium-glutathione peroxidases (SeGPXs), respectively (Rahman 2007). These cofactors are present at the enzyme catalytic site and their availability can determine the activity of such enzymes. SODs act to convert two superoxide radicals into hydrogen peroxide (H_2O_2) and oxygen. The family GPX catalyzes the reduction of H_2O_2 to water or lipid hydroperoxides to alcohol. Like GPX, catalase (CAT) decomposes H_2O_2 to water and oxygen. Although selenium is not itself an antioxidant, inadequate selenium is thought to adversely affect GPX activity (Fujii et al. 2005). Most of the GPX isoforms in mammals contain selenium and are dependent upon selenium for their function (Brigelius-Flohé and Maiorino 2013).

5.4 Antioxidants in Normal Reproductive Physiology

There is increasing evidence that antioxidants play a vital role in reproductive health and fertility. The local cellular environment influences oocyte development, ovulation, successful fertilization, and maintenance of early pregnancy. ROS and antioxidants have emerged as ubiquitous participants in normal human reproductive processes.

5.4.1 Puberty and Menarche

The age of puberty has decreased over the past 150 years creating much speculation into the etiology of the change, which is likely multifactorial. The decline has slowed or plateaued since the 1960s in the Western world; however, a decline is now being seen in some developing countries (Pierce and Hardy 2012). One hypothesis is that there has been a change in nutritional intake among today's population. From a pregnant woman's intake affecting fetal development, through lactation and the effects on breastfed infants, to the relatively recent expansion in soy-based infant formulas, to the changes in childhood food consumption and the explosion of childhood overweight and obesity, there are literally endless points along the spectrum of reproductive development that are subject to influence. Attention has gone into the effects of soy and soy products on reproductive development, as soy and the isoflavones it contains have known estrogenic properties and are able to interfere with the hypothalamic-pituitary axis.

Studies have reported conflicting results regarding phytoestrogen exposure and timing of puberty. A recent prospective longitudinal study (Adgent et al. 2012) found that soy-formula-fed infants experienced earlier menarche in comparison to breast-fed or other types of formula-fed infants. In this study, white females with term singletons were enrolled during pregnancy, and infants (n = 2124) fed soy formula (approximately 6–9 mg/kg/day isoflavone intake, with plasma levels reaching up to 1000 ng/mL (Patisaul and Jefferson 2010) at or before 4 months of age)

experienced a median age at menarche of 12.4 years compared to 12.8 years in the general study population (n = 2920) and the exclusively breast-fed population (n = 631) (Adgent et al. 2012). Another study (Zung et al. 2008) showed a significantly higher prevalence of breast buds in the second year of life in children fed soy formula, while a study by Bernbaum et al. showed re-estrogenized vaginal epithelium at 6 months of age in soy formula-fed infants in comparison to human or cow's milk-fed counterparts, whose vaginal epithelium did not re-estrogenize (Bernbaum et al. 2008). A Korean case-control study of approximately 200 girls (Kim et al. 2011), both with central precocious puberty (CPP) and age-matched controls, reported a significantly higher prevalence of CPP in girls with total serum isoflavone levels ≥30 nmol/L than those with levels <30 nmol/L.

In contrast, phytoestrogen exposure later in life, from food rather than formula, has been associated with delayed markers of puberty. A German longitudinal study of over 200 healthy children (Cheng et al. 2010) reported that girls with the highest levels of dietary isoflavones (423.4–19,178 μg/day) experienced onset of breast development and reached peak height velocity approximately 7–8 months later than those girls with the lowest intakes (4.1–21.9 μg/day). Adding evidence to dietary isoflavones' ability to delay breast development, a different German study (Cheng et al. 2012) reported that girls with the highest tertile intake of isoflavones (≥423 μg/day) experienced onset of Tanner stage 2 breast development 0.7 years later than girls with isoflavone intake in the lowest tertile (≤22 μg/day).

Jefferson et al. studied the effects of genistin (the glycosylated form of genistein and the substance that contributes >65% of the isoflavone content of soy formulas) in the reproductive health of rats. They found that supplementation with oral genistin (20, 40, and 60 mg/kg/day via genistein-equivalent doses, 6.25 mg/kg/day genistein = 10 mg/kg/day of genistin) resulted in altered ovarian differentiation (multioocyte follicles), delayed vaginal opening, abnormal estrous cycles, decreased fertility, and delayed parturition compared to control rats who were not supplemented. There was a positive correlation between dose and magnitude of effect. For comparison, infants fed soy formula are estimated to consume 4–7 mg/kg/day of genistein (Jefferson et al. 2009). Although the doses administered in the study are higher than those generally consumed by infants on soy formula, the pharmacokinetics of genistein in infants are not known, and further research is needed to determine health effects based on the dose and timing of exposure.

Outside of studies involving soy products, other studies have focused on the effects of vitamins and minerals on puberty, and there has been conflicting evidence. Higher consumption of dietary vitamin A (in the form of both retinol and beta-carotene) by 10–14-year-old girls has been associated with both a lower (Maclure et al. 1991) and higher (Moisan et al. 1990) age at menarche. A study of British girls (Rogers et al. 2010) found that those with higher intakes of magnesium or zinc were associated with an earlier age at menarche, while those with higher thiamin or iron intakes experienced later menarche (Kissinger and Sanchez 1987). It is unknown whether these effects can be attributed specifically to the vitamins' and minerals' antioxidant properties.

5.4.2 Menstrual Cycle

The normal female reproductive cycle is orchestrated by the interplay of various hormones, mainly luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, and progesterone, on the hormone-sensitive reproductive organs, ovaries, and uterus. There have been few studies examining the natural fluctuations in antioxidants across the normal menstrual cycle, and those that have been completed may lack the power to show significant differences within individuals. One study examining nine micronutrients, six lipids and lipid peroxidases, and antioxidant enzymes including GPX, SOD, and paraoxonase failed to show significant differences within nine healthy women across the menstrual cycle; however, it did show significant variation between individuals (Browne et al. 2008).

In contrast, Cornelli et al. did report variation in oxidative stress in individual women across the menstrual cycle (Cornelli et al. 2013). They found that the level of oxidative stress, as measured by the reactive oxygen metabolites test (d-ROMs), which measures hydroperoxide plasma levels and is used as a representative measure of oxidative stress, increases a few days before the estrogen peak and lasts through the progesterone phase, suggesting that a woman is in a state of relative oxidative stress for two-thirds of the normal menstrual cycle. Similarly, Michos et al. reported a steady increase in the total antioxidant capacity, alongside the increase in estradiol concentration, from the time of menstruation to ovulation in healthy, eumenorrheic Greek women (Michos et al. 2006). A Polish study of 12 women (Karowicz-Bilinska et al. 2008) also reported increased oxidative stress during the luteal phase. In their study, urinary H_2O_2, which has been proposed as a noninvasive marker for whole-body oxidative stress, was analyzed on each day of the menstrual cycle. Despite variations between individuals, a consistent pattern emerged within individuals: the level of H_2O_2 was found to be significantly lower across the entire follicular phase as compared to the luteal phase (Karowicz-Bilinska et al. 2008), suggesting that whole-body oxidative stress is increased during the luteal phase of the menstrual cycle.

5.4.3 Ovarian Function

Ovarian follicular development is dependent upon a number of mechanisms. The finite pool of primordial follicles must mature into the primary, preantral, and antral stage follicles and then finally reach the preovulatory stage and release during ovulation. Decreased levels of antioxidant enzymes have been reported in the follicular fluid of women with unexplained infertility, and ROS are known to be detrimental to oocyte quality (Tamura et al. 2008). ROS are involved in the follicular fluid environment, folliculogenesis, and steroidogenesis. Oocyte maturation is affected by ROS and antioxidants such as SOD1, SOD2, and GPX, which has been illuminated by immunohistochemical localization, mRNA expression studies, and thiobarbituric acid localization (Agarwal et al. 2006).

Unlike most organs, the function of the ovary is significantly influenced by ovarian and follicular development in utero. Bernal et al. reported that maternal undernutrition could have long-lasting and profound effects on the reproductive potential of offspring (Bernal et al. 2010). The process is unclear, though there is some thought that leptin acts as a metabolic signal to the central reproductive axis, acting as a permissive neuro-regulatory factor for the onset of puberty, and both stimulating and inhibiting ovulatory processes and ovarian steroidogenesis. It appears that maternal undernutrition may also increase oxidative stress in the developing fetus and offspring. In a study of rats (Bernal et al. 2010), maternal caloric undernutrition (rats fed 50% of a standard control diet) during pregnancy and lactation was shown to significantly reduce the primary and secondary follicle counts in their offspring. Antral follicle counts were reduced significantly in offspring of mothers undernourished during any stage of pregnancy and/or lactation. These offspring were found to have increased ovarian protein carbonyls and reduced levels of peroxiredoxin 3 hyperoxidation mRNA (a mitochondrial antioxidant protein that breaks down endogenous hydroperoxides), implying decreased antioxidant defenses and increased oxidative damage. It is speculated that oxidative stress accelerates reproductive maturity along with faster decline in ovarian reserve (Bernal et al. 2010).

Isoflavones have also been shown to modulate the normal reproductive cycle. A recent meta-analysis of 47 studies (Hooper et al. 2009) revealed that in premenopausal women, consumption of soy isoflavones in intervention groups reduced circulating LH and FSH and increased menstrual cycle length as compared to controls. However, subgroup analyses by isoflavone intake level (<25 mg/day, 25 to <50 mg/day, 50 to <75 mg/day, 75 to <100 mg/day, ≥100 mg/day) showed no significant effect. In a review of almost 50 studies on premenopausal women given soy supplementation (Jefferson 2010), no effect was seen on estradiol, estrone, or sHBG, but there was a decrease in both LH and FSH and increased cycle length in those supplemented with soy. In contrast, a prospective cohort study of more than 250 healthy menstruating women (Filiberto et al. 2013) reported that isoflavone intake was not found to be associated with estradiol, free estradiol, progesterone, LH, or FSH concentrations, but isoflavone intake in the highest quartile (1.6–78.8 mg/day) was significantly associated with a greater sHBG concentration, in comparison with consumption in the lowest quartile (0.0–0.3 mg/day). Isoflavone intake was not associated with sporadic anovulation in this cohort. This study suggests that, though isoflavones may cause some endocrine effects, they may not interfere with ovulation. Of note, most studies have shown that there are no significant effects resulting from soy consumption at normal dietary levels (10–25 mg soy isoflavones/day, and even up to 50 mg/day), but the effects of ovulatory hormone (e.g., LH, FSH) disturbance are fairly consistent with higher levels (e.g., ≥100 mg soy isoflavones/day, a level that can be achieved through diets very high in soy) (Jefferson 2010). In one of the only studies to show hormonal disruption resulting from low doses of isoflavones (Faber and Hughes 1993), low doses of genistein (10 μg subcutaneous genistein) were shown to increase GnRH-induced LH release in rats, while high doses (100–1000 μg subcutaneous genistein) decreased release of LH.

5.4.4 Uterine Function

Cyclic changes in the endometrium are accompanied by cyclic variations in the expression of antioxidants. For instance, both SOD1 and SOD2 are highly expressed in the epithelial and stromal cells in the endometrium, and SOD1 and SOD2 expression increases consistently throughout the proliferative phase and into the mid-secretory phase, where it is at similar levels to those found during early pregnancy. SOD1 and SOD2 activity decrease, while lipid peroxide levels increase, just prior to menstruation. The shift in favor of oxidative stress leads to production of $PGF2_{\alpha}$, which causes endometrial shedding via vasoconstriction (Sugino 2007). Thioredoxin (TRX), another antioxidant, is expressed in the early secretory phase, and it has been shown in mice that the targeted disruption of TRX, results in lethal effects on an embryo (Agarwal et al. 2006).

Isoflavones have been a topic of interest with regard to their estrogenic effects on the endometrium. One randomized controlled trial (Unfer et al. 2004a) demonstrated a significant increase in the incidence of endometrial hyperplasia (3.8% vs 0%) in 179 women randomized to soy tablets (150 mg/day of isoflavones) for 5 years as compared to placebo. This intake can be readily achieved through diet alone and is about half the intake in a traditional Asian diet. One manifestation of focal endometrial overgrowth is in the formation of endometrial polyps, which consist of a vascular core surrounded by localized pedunculated overgrowths of endometrial stroma and glands. Polyp formation is poorly understood and it is unknown why some women form polyps while others do not. One study (Pejić et al. 2013) examining the fluctuations in antioxidant enzyme levels within polyps in concert with fluctuations in reproductive hormone levels found a negative correlation between LH and SOD (SOD1 and SOD2), and between FSH/LH and glutathione peroxidase in polyp tissue, while in the blood there was a positive correlation between estrogen and SOD levels. Similar to prior studies, Pejic et al. found lower levels of lipid hydroperoxides (LHP) in the blood during the luteal phase when compared to the follicular phase (Pejić et al. 2013).

Uterine leiomyomas, also called fibroids or myomas, are benign muscle tumors of the uterus; they contain a large amount of extracellular matrix, feeding vessels, and smooth muscle fibers, and are surrounded by a thin capsule of areolar tissue (Santulli et al. 2013). Suggested they can cause heavy bleeding and pelvic pain and are associated with infertility and adverse birth outcomes. The pathogenesis of fibroids is largely unknown. A large prospective cohort study involving 19,972 women (D'Aloisio et al. 2010) suggested that women who self-reported being fed soy formula in infancy had an increased risk of developing leiomyoma later in life (RR = 1.25; 95% CI 0.97–1.61). A case-control study (Santulli et al. 2013) involving women undergoing surgery for leiomyoma and controls undergoing surgery for various other pelvic pathologies, found a significantly higher level of serum protein carbonyl groups and advanced oxidation protein products (AOPP) in the fibroid group compared to controls, while serum thiol (an antioxidant) levels were lower. Interestingly, the serum AOPP levels were positively correlated with the fibroid weight, and serum AOPP and serum protein carbonyls were positively correlated with the duration of infertility.

5.4.5 *Pregnancy*

The corpus luteum is a transient organ whose function is to produce progesterone when fertilization occurs in order to maintain the pregnancy. The deterioration of the corpus luteum in the absence of pregnancy is thought to be due to ROS-mediated events, while evidence suggests that antioxidant enzymes play a role in corpus luteum rescue from apoptosis when pregnancy ensues (Al-Gubory et al. 2012). Antioxidants likely play a vital role in the function of the corpus luteum (Arianmanesh et al. 2011); however, the particular antioxidants involved in its maintenance have not yet been established.

The pathway from fertilization through implantation and continued embryonic and fetal development is dependent upon innumerable factors. The role of antioxidants in various steps along the way has been studied with inconclusive results. In a study of 99 New York state women attempting conception (Bloom et al. 2011), blood metals were measured to determine whether there was a relation between levels of arsenic, cadmium, lead, magnesium, nickel, selenium, and zinc and conception. There were no statistically significant differences found. The authors observed a 51.5% increase in the probability of conception for every 3.60 μg/L increase in serum magnesium level ($\beta = 4.197$, 95% CL β −0.216, 8.610, $p = 0.062$), while there was a 27.7% decrease in the probability of conception for every 0.54 μg/L increase in zinc level ($\beta = -2.234$, 95% CL β −5.004, 0.536, $p = 0.114$).

Pregnancy is a physiologic state characterized by increased metabolic demands and requirements for oxygen, which increases the rate of production of ROS. In comparison to age-matched, nonpregnant women, women even with uncomplicated pregnancies have elevated levels of oxidative stress and of circulating lipid peroxides (Aversa et al. 2012). At the same time, Aversa et al. report that levels of many antioxidants such as vitamin E, erythrocyte thiols, ceruloplasmin, and iron-binding capacity are increased, and levels of SOD and GPX have been shown to decrease in pregnancy relative to nonpregnant levels. Fugii et al. hypothesized that SOD1 helps to maintain pregnancy by preventing the accumulation of superoxide radicals, thereby decreasing synthesis of $PGF_{2\alpha}$, which has been shown to increase at the end of spontaneous abortion (Fujii et al. 2005). Although both oxidative stress and some antioxidant levels increase throughout pregnancy, the overall balance appears to sway in favor of elevated ROS (Aversa et al. 2012).

5.5 Antioxidants in Reproductive Disorders and Complications

ROS-induced cellular oxidative stress can adversely affect function of reproductive organs and fertility. Imbalance in the oxidant/antioxidant relationship has been implicated in numerous reproductive disorders and complications, including endometriosis, polycystic ovarian syndrome, oocyte aging, dysmenorrhea and premenstrual syndrome, spontaneous abortion, and infertility (Fig. 5.1).

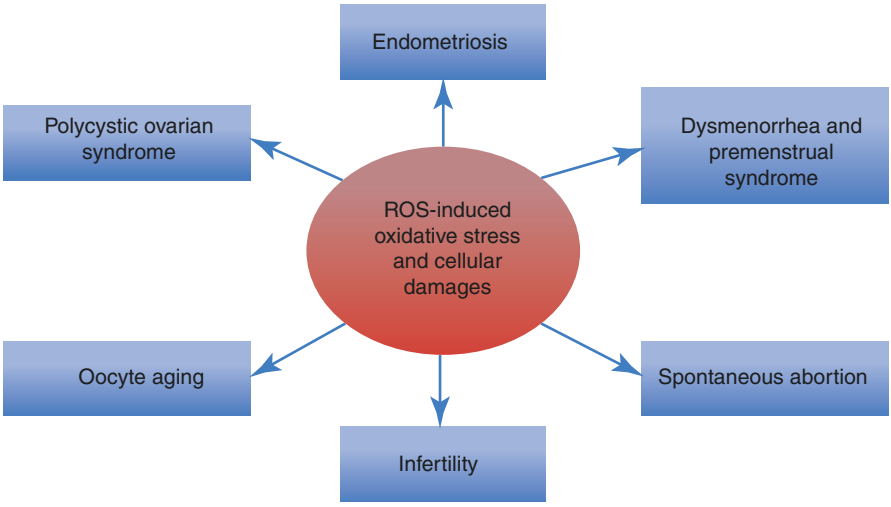

Fig. 5.1 Reactive oxygen species (ROS)-induced cellular oxidative stress and reproductive disorders and complications

5.5.1 Endometriosis

Endometriosis is characterized by implantation of endometrial tissue outside the uterus and is a chronic inflammatory disease that affects 21–44% of infertile women and 4–22% of fertile women (Tamura et al. 2009), (Fig. 5.2). This disease manifests symptomatically with chronic pelvic pain, progressive dysmenorrhea, and dyspareunia. Women with endometriosis have been shown to increase peritoneal fluid production, along with increased peritoneal fluid macrophage production of ROS (Tamura et al. 2009). This leads to a localized inflammatory reaction favoring oxidative stress; it is unknown whether the relative state of oxidative stress leads to, or is a result of, endometriosis.

A lower total antioxidant potential has been reported in the pelvic fluid and serum of infertile women with endometriosis as compared to infertile women without endometriosis. In a study on serum ROS markers in women undergoing pelvic laparoscopy for a variety of indications (Jackson et al. 2005), those who were diagnosed with endometriosis at the time of laparoscopy had a weak association with thiobarbituric acid-reducing substances (TBARS), a measure of lipid peroxidation. In a cohort study (Prieto et al. 2012) of 91 women with infertility, four markers of oxidative stress were studied in serum and follicular fluid samples obtained at the time of egg retrieval. Women with infertility due to endometriosis (n = 23) were shown to have lower levels of follicular fluid vitamin C, lower plasma SOD concentrations, and lower plasma vitamin E levels than women with infertility due to other reasons, who served as the controls (n = 68). The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde (MDA). There was a nonsignificant trend toward lower serum MDA levels in women with endometriosis. Jana

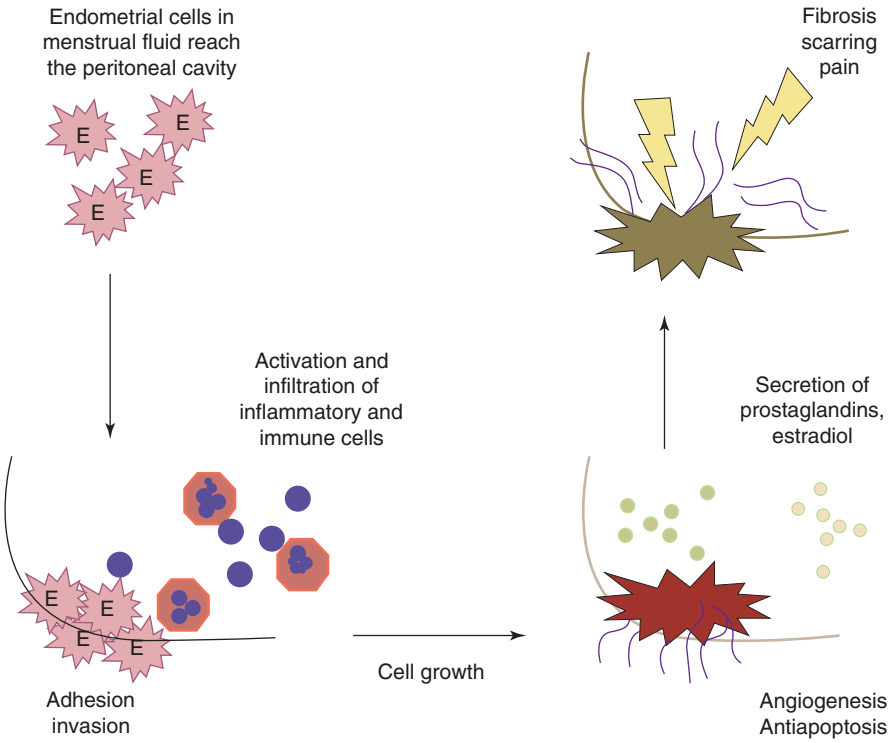

Fig. 5.2 A model of disease for endometriosis based on data from both animal and human studies (reprinted with permission from Flores et al. 2007)

et al. studied 135 women with infertility and similarly reported that those with endometriosis had higher levels of ROS, lipid peroxidation, and advanced oxidation protein products and lower levels of total antioxidant capacity, SOD, CAT, and glutathione than the controls with tubal factor infertility (without endometriosis). This study used proton nuclear magnetic resonance spectroscopy based on targeted metabolite profiling (Jana et al. 2013).

Not only the overall concentrations of antioxidants but also the fluctuations throughout the menstrual cycle have been studied in the context of endometriosis. Women with endometriosis have been shown to have consistently elevated levels of xanthine oxidase, SOD, CAT, and GPX expression throughout their cycles, whereas women without endometriosis have cyclic variations in these enzyme levels (Gupta et al. 2006). This may suggest that there is a consistently increased ROS burden in the setting of endometriosis, with a concomitant rise in antioxidant production in an attempt to combat oxidative damage. Once the importance of oxidative stress in the pathophysiology of endometriosis was discovered, studies involving interventions with antioxidant supplementation began to appear with some promising results, first in laboratory studies and animal models and later in humans. One study of human

endometriotic and healthy endometrial cells both in vitro and implanted into mice (Ngô et al. 2009) showed increased ROS production, altered ROS detoxification pathways, and decreased catalase levels in endometriotic cell lines. Addition of the antioxidant, *N*-Acetyl-Cysteine (NAC), to the cultured cells in vitro and in mouse models seemed to counterbalance the oxidative stress markers in the endometriotic cell lines.

Resveratrol, a natural polyphenol synthesized by plants in response to ultraviolet radiation and fungal infections, has been studied for its antioxidant properties in a variety of conditions. In a mouse study, supplementation with resveratrol following injection of human endometriotic implants was shown to decrease the number of implants by 60% and the total volume by 80% compared to controls (Goud et al. 2008). The invasiveness of human endometrial stromal cells in culture was also decreased significantly with the addition of resveratrol, by up to 78%, in a concentration-dependent fashion compared to controls. In a similar study of resveratrol in mice (Rudzitis-Auth et al. 2013), supplementation with resveratrol was shown to inhibit angiogenesis in peritoneal and mesenteric endometrial implants along with reducing growth and resulting in lower final size of implants in comparison to controls not given resveratrol. Resveratrol is a promising antioxidant therapy for endometriosis and deserves further study.

As the long-term survival of endometriotic implants is dependent upon neovascularization, it has been postulated that high concentrations of vascular endothelial growth factor (VEGF) permit the growth of vasculature necessary for the survival of these implants. High concentrations of VEGF have been found in the peritoneal fluid of women with endometriosis (Chaudhury et al. 2013). Therefore, recent studies have focused on anti-angiogenic strategies for treatment of endometriosis. Nanoceria, or cerium oxide nanoparticles, have been studied in a variety of diseases for their antioxidant and anti-inflammatory properties. These particles can mimic SOD and CAT. In a study of mice injected with mouse endometrial implants (Chaudhury et al. 2013), those treated with nanoceria were found to have decreased levels of oxidative stress and angiogenic factors, along with decreased endometrial glands, in comparison to controls or those mice treated with NAC. Mice treated with nanoceria also had a higher number and quality of oocytes compared to control or NAC-treated mice. In another mouse study (Jana et al. 2012), curcumin, a known antioxidant and anti-inflammatory constituent of turmeric, was found to cause regression of endometriosis by inhibiting NF-kB translocation and matrix metalloprotein-3 (MMP-3) expression and to accelerate apoptosis in endometrial implants by a cytochrome-c mediated mitochondrial pathway. MMPs support the growth and invasion of endometrial implants and are modulated by NF-kB. In this study, curcumin upregulated p53 expression by about tenfold on Western blot densitometry (signal intensity 0.8 as compared to 0.1 in the control group) and the numbers of peritoneal endometrial glands were found to be decreased about threefold on densitometry (signal intensity of approximately 4 as compared to 12) by pretreatment with curcumin as compared to controls.

Vitamins C and E have been studied to improve endometriosis at both cellular and clinical levels. In a randomized, placebo controlled trial of 59 women with pelvic pain and a history of endometriosis and/or infertility (Kavtaradze et al. 2003), 43% of those in the intervention group (given 1200 IU of all-racemic vitamin E and 1000 mg of vitamin C daily for 2 months), reported a significant improvement in chronic daily pain as compared to the placebo group, who reported no significant improvement. Though not statistically significant, there was clinical improvement in dysmenorrhea and dyspareunia, with 37% reporting significant improvement in dysmenorrhea and 24% reporting significant improvement in dyspareunia compared to controls. Another study (Mier-Cabrera et al. 2008) randomized infertile women with endometriosis to 343 mg vitamin C and 84 mg all-racemic vitamin E versus placebo for 6 months. They found that plasma and peritoneal fluid levels of MDA and lipid hydroperoxidases were significantly decreased by 6 months in the intervention group as compared to the placebo group. Of note, the post-intervention pregnancy rates (19% in intervention group vs. 12% in placebo group by 9 months post-study) were not statistically significantly different.

5.5.2 Polycystic Ovarian Syndrome

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder of reproductive-aged women and also a common cause of infertility. It is characterized by anovulation, androgen excess, and insulin resistance (Fenkci et al. 2003). Like other reproductive pathologies, PCOS is likely to be partially caused by, and certainly results in, imbalance of oxidants and antioxidants. In a case-control study of 30 women with PCOS and 31 healthy age-matched controls (Fenkci et al. 2003), antioxidant status was measured in serum samples using 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate acid (ABTS)) radical cation assay. Total antioxidant status was lower in women with PCOS than in controls. In another case-control study of 33 women with PCOS and 28 healthy controls (Hilali et al. 2013), serum prolidase, total oxidant status (TOS), as measured by the an automated method (Erel 2005), oxidative stress index (as measured by the percent ratio of TOS to total antioxidant status), LH, prolactin, and testosterone levels were significantly higher in women with PCOS as compared to controls, and total antioxidant status (TAS), measured by another automated method (Erel 2004) was lower in women with PCOS, however this difference was not statistically significant. In a Turkish study (Coskun et al. 2013) examining serum levels of selenium in 36 women diagnosed with PCOS and 33 age and body mass index-matched healthy controls, women with PCOS were found to have significantly lower plasma selenium levels than controls. There was also a statistically significant negative correlation noted between selenium level and LH and total testosterone level in all women studied. PCOS, along with other reproductive pathologies, is an area that would benefit greatly from further antioxidant research.

5.5.3 Oocyte Aging

Accumulating data suggest that ROS deteriorate oocyte quality and accelerate oocyte aging (Tamura et al. 2012). In order to protect the fragile oocytes from oxidative damage, antioxidants must be present in adequate concentrations. Oocyte senescence is thought to be at least partially modulated by an increase in oxidative stress, as a reduction in glutathione and catalase activity and an increase in SOD have been found in older women compared with younger women (Agarwal et al. 2006). It is unknown whether antioxidant defenses within the ovary decrease with age or whether the ROS concentration increases such that significant damage is caused to remaining oocytes, resulting in senescence. In a study of rat oocytes (Goud et al. 2008), young oocytes were resistant to H_2O_2 effects, while relatively "old" oocytes experienced accelerated aging when exposed to H_2O_2. When exposed to low levels of hypochlorous acid, young oocytes experienced accelerated aging, while lysis was seen in "old" oocytes. Furthermore, at high concentrations, oocyte viability was compromised in both age groups. This suggests that young oocytes have enhanced antioxidant defenses as compared to "old" oocytes. In fact, antioxidants such as vitamin C and reduced glutathione have been shown to be protective against postovulatory oocyte aging in vitro (Goud et al. 2008).

5.5.4 Dysmenorrhea and Premenstrual Syndrome

Antioxidant status has been studied in women with painful menses and premenstrual syndrome. In a study of 20 healthy women (Duvan et al. 2011), with clinically diagnosed premenstrual syndrome (PMS) and 21 controls, serum oxidant and antioxidant status was evaluated by measurement of lipid hydroperoxides, MDA, protein carbonyl, total thiol (T-SH), and total antioxidant capacity (TAC), measured by the ferric reducing ability of plasma (FRAP) assay via serum samples on day 3 and day 21 of the menstrual cycle. The PMS group was found to have statistically increased day 21 lipid hydroperoxides and decreased day 3 and day 21 TAC levels in comparison to the control group, suggesting that those with PMS have an increase in oxidative stress throughout the menstrual cycle.

Thiamin (vitamin B1) acts as an antioxidant (Lukienko et al. 2000) and has been studied as an intervention for dysmenorrhea. In an Indian crossover study of 556 women with dysmenorrhea (Gokhale 1996), 100 mg of thiamin daily for 3 months resulted in improvement in dysmenorrhea in >90% of women, compared to <1% of controls taking a placebo. There have been a few studies examining vitamin E supplementation for dysmenorrhea and menorrhagia with promising results (Ziaei et al. 2001, 2005; Butler and McKnight 1955). Although the mechanism of action is unclear, it is thought that vitamin E might inhibit arachidonic acid release and thereby decrease prostaglandin formation (Ziaei et al. 2005). Magnesium, vitamin B6, and omega-3 fatty acids have also been studied for dysmenorrhea with mixed results (Dennehy 2006). These areas would benefit from further study.

5.5.5 *Infertility*

As has been shown with other reproductive functions, fertility can be affected by changes in the oxidant/antioxidant balance. Much research has gone into determining the causes behind infertility, and oxidative stress has been a frequent topic of both basic science and clinical studies (Ruder et al. 2008), (Fig. 5.3). Fairly consistently across studies, women with unexplained infertility have decreased antioxidant enzyme levels in their follicular fluid (Tamura et al. 2012). More specifically,

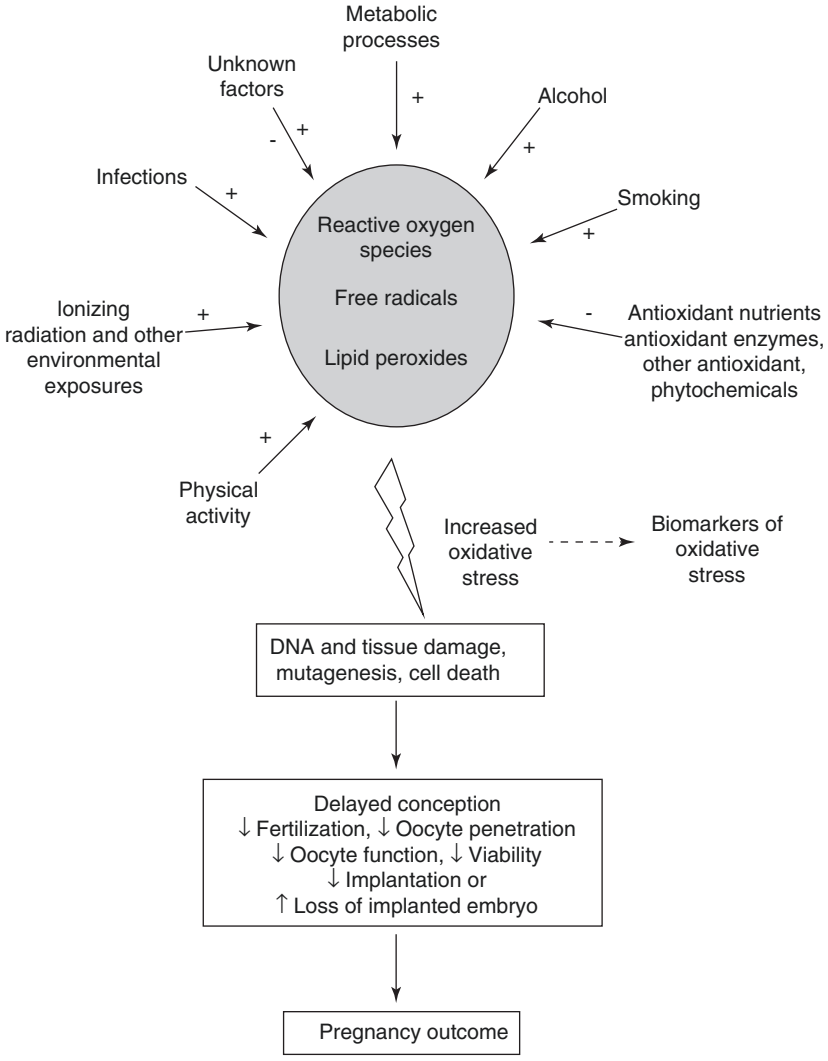

Fig. 5.3 The role of oxidative stress in fertility (reprinted with permission from Ruder et al. 2008)

follicular fluid from women with unexplained infertility was found to have decreased selenium content than in those women with tubal infertility or a known male-related cause (0.44 μmol/L vs. 0.51 μmol/L, $p < 0.05$) (Paszkowski et al. 1995). Women with unexplained infertility or premature ovarian failure have also been shown to have significantly higher serum levels of the autoantibody protein, selenium-binding protein-1 (Edassery et al. 2010). Increased oxidative stress alters the fatty acid profile due to peroxidative breakdown. As fatty acids are the precursors to prostaglandins, which mediate various reproductive processes, it is postulated that altered fatty acid profiles can have a direct effect on fertility. An Indian study (Mehendale et al. 2009) found significantly reduced fatty acid levels (eicosapentaenoic acid and erythrocyte docosahexaenoic acid) and increased levels of MDA along with decreased levels of vitamin E, in the serum of infertile women as compared to controls.

Black cohosh, a plant with conflicting evidence for its estrogenic properties, and other supplements have also been studied. Women with unexplained infertility undergoing ovulatory induction with clomiphene who were supplemented with black cohosh (120 mg/day for cycle days 1–12) were found to have increased levels of LH, progesterone, and estradiol, increased endometrial thickness, and increased pregnancy rates compared to controls (Shahin et al. 2008). In another study (Unfer et al. 2004b), women who were supplemented with luteal phase support using progesterone (50 mg daily) and phytoestrogen tablets (1500 mg soy isoflavone tablet daily containing 40–45% by weight genistein, 40–45% by daidzein, 10–20% glycitein) had significantly higher implantation, clinical pregnancy, and delivery rates compared to controls. A randomized controlled trial of 150 women with luteal phase defects (Dennehy 2006) found that supplementation with 750 mg/day of vitamin C resulted in a significantly higher fertility rate as compared to the placebo (25% vs. 11%); progesterone levels were also significantly increased in the treatment group (52.6% vs 21.7%).

Although the mechanism by which ROS affect fertility is not yet clear, elevated levels of ROS and inflammatory cytokines have been shown to reduce ciliary beat frequency in fallopian tubes (Comhaire 2010). This will surely be an area of continued research, as minimally invasive interventions to improve fertility have the potential to drastically reduce patient morbidity and cost to both patients and the healthcare system.

5.5.6 Spontaneous Abortion

The etiology of spontaneous abortion is, more often than not, unknown. Although many spontaneous abortions are due to chromosomal defects, environmental factors also play a large role, and the oxidant/antioxidant balance is likely to be a factor in the pathophysiology behind, or sequelae of, spontaneous abortions. Biochemical markers of ROS-induced membrane damage, such as lipid peroxidation products, reach very high levels immediately prior to spontaneous abortion (Gupta et al. 2007). While increased levels of antioxidants have been demonstrated in normal,

healthy pregnancies, loss of antioxidant defenses has been seen in women with recurrent abortion (Şimşek et al. 1998).

Much research has focused on selenium status in women with spontaneous abortion, and results have been conflicting. A statistically significant decrease in serum or red blood cell selenium levels was found in women with a history of recurrent pregnancy loss in two studies, as compared to healthy nonpregnant women with at least one successful pregnancy and no history of spontaneous abortion (Kumar et al. 2002; Koçak et al. 1999). An observational study in the United Kingdom (Mistry et al. 2012) reported significantly lower serum selenium concentrations in women with first trimester spontaneous abortion in comparison to age-matched nonpregnant or healthy gestation-matched women. A case-control study in Indonesia (Abdulah et al. 2013) similarly reported significantly decreased serum selenium concentrations in women who had undergone spontaneous abortion compared with controls; glutathione peroxidase levels were similar in the two groups. However, two other studies reported no differences in serum selenium between women with recurrent spontaneous abortion and healthy women with no history of spontaneous abortion (Al-Kunani et al. 2001; Nicoll et al. 1999). Aberrant placentation has also been implicated in recurrent spontaneous abortion (Burton et al. 2003), and it is thought that this leads to increased oxidative stress and syncytiotrophoblast dysfunction, a proposed cause of spontaneous abortion. Gupta et al. reported elevated plasma levels of lipid peroxides and glutathione, as well as lower levels of vitamin E and β-carotene in women with spontaneous abortion (Gupta et al. 2007).

Few studies have yet attempted interventions with antioxidants during the periconception and early prenatal period. However, an Egyptian prospective study (Amin et al. 2008) enrolled 166 women with a history of recurrent unexplained pregnancy loss and treated one group with NAC (0.6 g) and folate (500 μg/day) and a control group solely with folate (500 μg/day). The intervention group with NAC + folate experienced a significantly higher rate of continuation of a living pregnancy beyond 20 weeks (RR 2.9; 95% CI 1.5–5.6) and also take-home baby rate (RR 1.98; 95% CI 1.3–4.0). Although antioxidant supplementation may hold promise in terms of increasing rates of conception and pregnancy retention (Al-Gubory 2013), the difficulties in getting Human Subject Committee approval for intervention studies concerning pregnant women will likely make progress in this area slow.

There is great interest in antioxidant supplementation for women with unexplained infertility. In a secondary data analysis of 437 women with unexplained infertility participating in the Fast Track and Standard Treatment Trial (FASTT) (Reindollar et al. 2010), women with body mass index ≥25 kg/m^2 who had an increased intake of β-carotene from dietary supplements had a shorter time to pregnancy (HR 1.29, 95% CI 1.09–1.53 in continuous analyses adjusted for treatment, age, and total energy intake) (Ruder et al. 2014). Intake of vitamin C dietary supplements was also associated with a shorter time to pregnancy among women with body mass index <25 kg/m^2 (HR 1.09, 95% CI 1.03–1.15) and women <35 years old (HR 1.10, 95% CI 1.02–1.18). Vitamin E supplementation was associated with a shorter time to pregnancy in women ≥35 years old (HR 1.07, 95% CI 1.01–1.13). These data support the theory that age, body mass index, and antioxidant status combined contribute to fertility and offer avenues for further study.

5.6 Conclusions

Although there is a growing interest in the role of antioxidants in normal reproductive physiology and also their therapeutic role in various pathologies, there are many methodological issues that make conclusive data difficult to produce. There are numerous forms of most antioxidants, and the forms and dosages used in different studies are so varied that it is difficult to compare results. While animal models are an excellent place to start, more human studies are needed. However, as antioxidants are present in a variety of foods and supplements, it becomes difficult to control for these exposures when analyzing human data in observational studies. Therefore, randomized controlled trials are ideal but are rarely approved in the setting of human conception and pregnancy, an area that would benefit greatly from further study. The spectrum of normal oxidant and antioxidant levels, both in organs and in serum, is still unknown, and it is difficult to draw conclusions without a solid basis for comparison. Many more carefully conducted, controlled, and adequately powered studies are needed in this field of research.

Normal human physiology is dependent upon a fine balance between oxidants and antioxidants. The female reproductive system and fertility are complicated and dynamic topics, of which much is still unknown. As studies continue to emerge, it is becoming increasingly clear that there is a complex and multifaceted interplay between ROS and antioxidants in the normal healthy state and that pathology emerges when this balance is offset. Whether oxidative stress is causative to pathologic conditions or results from them is unknown; however, it is clear that conditions ranging from spontaneous abortion to endometriosis favor oxidative stress and interventions with antioxidants have the potential to slow, reverse, or prevent these conditions. Unfortunately, studies examining antioxidant supplementation have been inconclusive to date. There is a vast knowledge deficit to fill, and many more studies are needed to elucidate the role of antioxidants and oxidative status in normal reproductive physiology and fertility, as well as in reproductive pathology. Antioxidants in reproductive health and fertility are a subject in its infancy and will continue to unfold as researchers and clinicians study this intriguing topic.

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Chapter 6
Antioxidant Therapy in Assisted Reproductive Technologies

Ashok Agarwal and Damayanthi Durairajanayagam

Abstract Physiological levels of reactive oxygen species (ROS) are required for proper functioning of the male and female reproductive system. However, imbalances between ROS production and antioxidant systems induce oxidative stress, which can jeopardize the quality of the gamete and the developing embryo and cause many pregnancy disorders, such as spontaneous abortion, recurrent pregnancy loss, preeclampsia, fetal embryopathies, and intrauterine growth restriction. This review discusses the adverse effect of ROS-induced oxidative stress in assisted reproductive technologies (ART) outcome, ROS generated in vitro by gametes and embryos, and ROS generated by external sources in the in vitro fertilization (IVF) laboratory and the protocols used, including gamete/embryo handling, composition and pH of culture media, temperature and oxygen concentration during incubation, centrifugation and freeze-thaw protocols, as well as visible light. Studies on oral supplementation of enzymatic and nonenzymatic antioxidants are discussed. Although there is no one antioxidant that is considered the best choice for improving ART outcomes, some antioxidants show promising results. Additional well-designed trials are needed to determine the appropriate type(s) and concentration of antioxidant(s) that would be helpful to infertile patients with various etiologies. Studies are also warranted in the improvement of ART protocols to minimize ROS formation during assisted reproduction.

Keywords Reactive oxygen species • Oxidative stress • Antioxidant supplementation • Assisted reproduction technologies

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6.1 Introduction

Infertility is a globally prevalent issue that affects 10–15% of couples of reproductive age (Homan et al. 2007). In the United States of America, between 8% and 15% of couples are unable to conceive even after a year of regular, unprotected intercourse (Stephen and Chandra 2006). However, despite recent advances in the diagnosis and treatment of reproductive disorders, about 15–30% of infertile couples have normal test results upon infertility evaluation and are deemed to have unexplained infertility (Quaas and Dokras 2008). Couples with fertility issues can turn to assisted reproduction technologies (ART) in order to conceive. In the United States of America, the use of ART has doubled over the past 10 years, and more than 1% of all babies born in each year are conceived through assisted reproduction (CDC 2014). However, achieving a pregnancy and carrying the fetus to live birth do not come easily to most couples seeking assisted reproduction, especially in women of advanced reproductive age. For example, according to the Society for Assisted Reproductive Technology's 2012 ART Clinic Summary Report, the number of embryo transfers that result in live births is 38% in women aged 35–37 years and 28% in women aged 38–40 years (SART 2014). Factors influencing the success of ART may stem from the patient themselves or from the quality of ART treatment. While ART, such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), attempt to simulate natural reproduction as closely as possible, there are many limitations of IVF compared to those achieved in vivo under physiological conditions. These limitations give rise to multiple factors that could compromise the success of ART, with one of the main culprits being the excessive production of reactive oxygen species (ROS). Therefore, therapeutic strategies such as antioxidant treatment of couples with fertility issues and/or antioxidant supplementation to culture media of ART could minimize the detrimental effects of ROS-induced oxidative stress. This would serve to defer ROS-induced influence on the gametes and the resulting embryo and ultimately increase the chances of a successful ART outcome. In this review, we will discuss the main source of oxidative stress in ART, ROS generated in vitro by gametes and embryos and by external sources in the IVF laboratory, as well as the role of antioxidant therapies in ART.

6.2 Effects of ROS on Gamete Quality and Embryo Development

ROS are either free radicals (oxygen molecules with one or more unpaired electrons) or non-radicals (oxygen molecules without any unpaired electrons). The primary form of ROS is the superoxide anion radical ($^{\bullet}O_2^-$), which can be converted into a secondary ROS such as the hydroxyl radical ($^{\bullet}OH$) or hydrogen peroxide (H_2O_2). Other examples of ROS include singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitric oxide ($NO^{\bullet}$), and the peroxynitrite anion ($ONOO^-$). ROS are formed

from biochemical reactions that occur within the mitochondrial respiratory chain (Tremellen 2008).

ROS are produced within the sperm mitochondria and plasma membrane (de Lamirande and Gagnon 1993a). High levels of ROS damage proteins, lipids, and nucleic acids (Riffo and Parraga 1996). Sperm with elevated levels of DNA fragmentation are associated with decreased in vitro fertilization rates (Sun et al. 1997). Infertile men with high ROS levels show higher levels of apoptosis in mature spermatozoa (Agarwal and Said 2003). The two main sources of ROS in human semen are spermatozoa and leukocytes, predominantly neutrophils (Tremellen 2008). In males, the sperm cell contains a limited amount of cytoplasm and therefore offers little protection by antioxidants. Seminal plasma, however, is rich in antioxidants, containing four enzymatic antioxidant systems: superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), and catalase (CAT) (Nenkova and Alexandrova 2013). Sperm that retain their residual cytoplasm due to faulty spermiogenesis (immature sperm) are intrinsic generators of ROS, while leukocytes are known to extrinsically produce large amounts of ROS (Kefer et al. 2009). The lipid peroxidation cascade affects sperm membrane fluidity, which in turn impacts sperm motility. Infertile men with high malondialdehyde (MDA) levels (a by-product of lipid peroxidation) were found to have poor in vitro fertilization rates (Ben Abdallah et al. 2009). In addition, increased ROS concentrations may impair the mitochondrial membrane potential, which may then lead to the activation of the apoptotic signaling pathway in sperm cells (Wang et al. 2003).

Generators of ROS in female are the preovulatory follicle, corpus luteum, ovaries, follicular fluid environment (oocyte, granulosa cells, endothelial and thecal cells), fallopian tubes, and the uterus (Pasqualotto et al. 2004; Gupta et al. 2008; Bedaiwy et al. 2012). At low concentrations, ROS are necessary for normal physiological processes such as sperm maturation, sperm capacitation and hyperactivation, acrosome reaction, and sperm-oocyte fusion (Kothari et al. 2010). Physiological levels of ROS are likely required for proper folliculogenesis, ovarian steroidogenesis, ovulation, oocyte maturation, and luteolysis (Gupta et al. 2008; Ruder et al. 2008). Antioxidants are endogenously present in the tubal fluid, endometrial epithelium, follicular fluid, and ovaries (Rakhit et al. 2013). Human follicles contain both enzymatic antioxidants (SOD, GPX, and CAT) and nonenzymatic antioxidants (glutathione, vitamins C and E, albumin, and uric acid) and melatonin, which is taken up from the blood into the follicular fluid (Tamura et al. 2014). High levels of ROS in the female reproductive system negatively affect oocyte and embryo development and quality, thus compromising the female reproductive potential (Agarwal et al. 2005). High ROS levels damage oocytes and reduce their quality by hastening the aging process (Goud et al. 2008). Oxidative stress increases cytoplasmic fragmentation in the embryo and causes apoptosis (Yang et al. 1998). ROS can also damage granulosa cells that are undergoing luteinization. High ROS levels impair DNA, causing poor embryo quality and lower fertilization rates (Seino et al. 2002). Table 6.1 summarizes the detrimental effects of oxidative stress on human gametes and embryos.

Table 6.1 Effects of high reactive oxygen species levels on the gametes, sperm-oocyte fertilization, and embryo development

Male gamete	Female gamete	Fertilization	
Spermatozoa	Oocytes	Sperm oocyte	Embryo development
Lipid peroxidation	Meiotic spindle damage	Reduced oocyte penetration	Mitochondrial alterations
DNA fragmentation	Poor oocyte quality	Lower fertilization rates	Embryo cell block
Apoptosis		Lower implantation rates	Depletion of ATP
		Higher early pregnancy loss	Reduced cleavage
			Apoptosis

6.3 Sources of ROS in Assisted Reproductive Technologies

In an in vitro setting, the protective effects of natural antioxidants are absent (Gupta et al. 2009). Hence, the risk of exposure is much greater than that in vivo (Agarwal and Allamaneni 2004). In vivo, the production of ROS is maintained at physiological levels by antioxidants in the male and female reproductive systems. The protection conferred by naturally occurring antioxidants is lost when fertilization is attempted outside the reproductive system. Figure 6.1 highlights the effects of oxidative stress during various steps in assisted conception and possible interventions to minimize these effects. High levels of ROS are more likely to be found in men with teratozoospermia or leukocytospermia, as sperm with abnormal morphology and a high seminal leukocyte count are known producers of ROS (de Lamirande and Gagnon 1993b). In fact, sperm that are used in about half of all IVF cases have already been exposed to oxidative stress prior to the IVF cycle (Saleh et al. 2003). During ICSI, it is more detrimental if the sperm selected had oxidative stress and its related consequences (e.g., DNA damage), as this sperm would be placed directly inside the oocyte, where it will pose a great risk for the developing embryo. In fact, high levels of ROS are negatively correlated with all ART outcomes such as fertilization rates, embryo development, and clinical pregnancies (Baker and Aitken 2005).

6.3.1 Gamete/Embryo Manipulation

The embryo cultured in vitro produces more ROS than those in vivo (Goto et al. 1993), raising the possibility that ROS produced during ART procedures can affect embryos development that are cultured in vitro. ROS production at higher than physiological levels can occur due to in vitro gamete and embryo manipulation in the clinical setting during ART (Lampiao 2012). This is more evident during IVF than ICSI. In an IVF procedure, during the long incubation period in the fertilization medium, the sperm and the oocyte, along with its cumulus cells, are

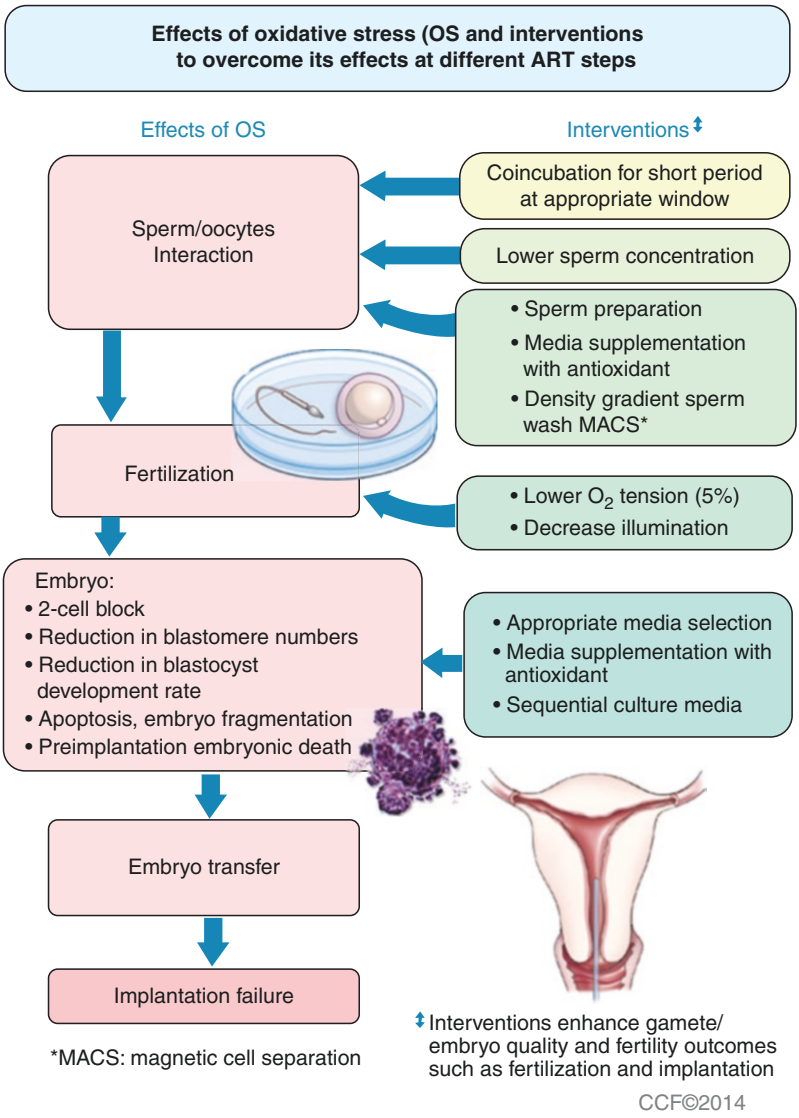

Fig. 6.1 Effects of ROS during ART and intervention

potential ROS generators. During ICSI, the cumulus cells are stripped away from the oocyte, thereby reducing the amount of ROS that could potentially be produced (Agarwal et al. 2006a). Further, a high concentration of sperm is used in an IVF procedure, whereas in ICSI, a single spermatozoon is used. Here again, the ICSI procedure has fewer potential producers of ROS, i.e., only the single spermatozoon and the denuded oocyte. Further, ICSI incubation times are shorter than those used in IVF. Thus, in an ICSI procedure, there is a comparatively lesser amount of incubation time during which the oocyte is exposed to abnormal spermatozoa, which therefore lowers the risk of ROS production (Rakhit et al. 2013).

In ICSI, the danger is more imminent from the probability of ROS-containing culture media being transferred along with the single spermatozoon into the oocyte, which consequently would expose the oocyte DNA to ROS-induced detrimental effects (Agarwal et al. 2003). Further, ICSI is usually the indication for cases where the sperm quality is very poor, and thus the selected spermatozoon, which may appear morphologically normal, may well be a sperm with defective DNA (Gandini et al. 2000).

6.3.2 Culture Media and Environment

The composition of culture media can directly influence the quality of the embryo produced and, subsequently, the success rate of the IVF procedure (Agarwal et al. 2006b). Certain components of culture media, such as metallic ions like iron or copper, can cause ROS generation (Guerin et al. 2001), while other additives such as serum albumin can increase the formation of H_2O_2 (du Plessis et al. 2008). Supplementation of culture media with antioxidants, e.g., ascorbic acid or alpha-tocopherol, can help lower the risk of oxidative stress (Sikka 2004). Culture media pH can affect sperm motility, oocyte maturation, and embryo development (Bagger et al. 1987; Will et al. 2011). In order to maintain the culture media pH, CO_2 levels in the incubator are maintained at a constant level. In addition, incubator temperature is maintained at 37 °C to mimic human body temperature (Suzuki and Mittler 2006). Heat stress caused by elevated temperatures could result in ROS-induced lipid peroxidation (Larkindale and Knight 2002). In ART laboratories, high concentrations of oxygen in the incubator environment increase the generation of ROS and consequently cause oxidative stress (Cohen et al. 1997). During IVF and ICSI cycles, embryos cultured at 5% oxygen were better developed and were associated with higher pregnancy rates than embryos cultured at 20% oxygen (Kovacic et al. 2010).

6.3.3 Centrifugation, Freeze-Thawing, and Light Exposure

During sperm preparation in ART, centrifugation is used to separate spermatozoa from the seminal plasma and motile sperm from immotile, dead sperm and other cellular debris (Agarwal et al. 2006a). The speed, duration, and temperature of centrifugation may impact sperm quality and ART outcomes at least in part through ROS generation (Shekarriz et al. 1995). Adding antioxidants such as pentoxifylline before centrifugation and during sperm preparation reduces ROS production (McKinney et al. 1996). Freeze-thaw procedures result in higher 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels (a marker of oxidized DNA damage) indicating greater DNA oxidative damage and DNA fragmentation levels in post-thaw sperm. This in turn leads to poor sperm motility and viability (Thomson et al. 2009; Zribi

et al. 2010). Adding antioxidants to sperm before freezing and during thawing could minimize the adverse effects of ROS generation during these processes. Gametes and embryos maintained in media during assisted reproductive procedures are exposed either to natural sunlight and/or artificial light prior to transfer. These types of light induce ROS within the cell (Girotti 2001), especially with prolonged exposure to microscope lighting (Ottosen et al. 2007).

6.4 Antioxidant Therapies

Antioxidants in an ART setting may be administered either into the culture media during the ART process so as to quell the formation of excessive ROS or given to the male and/or female partner several months prior to an ART cycle, in an attempt to counteract the damaging effects of excessive ROS production and thus improve the quality of the gametes. Figure 6.2 illustrates the classification of various types of common enzymatic and nonenzymatic antioxidants. Common dietary antioxidants include vitamins E and C, folate, coenzyme Q10 (CoQ10), trace elements, carotenoids, lycopene, and beta-carotene (Agarwal et al. 2014).

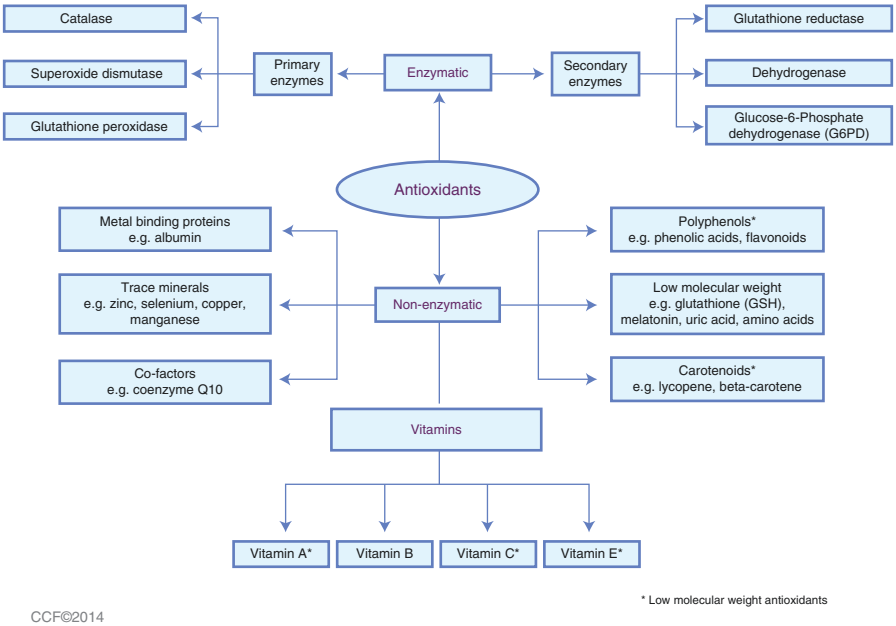

Fig. 6.2 Classification of antioxidants

6.4.1 Vitamin E

Vitamin E is a fat-soluble antioxidant compound that consists of four tocopherols and four tocotrienols (each alpha, beta, gamma, and delta). Vitamin E scavenges $\bullet O_2^-$, H_2O_2, and •OH (Agarwal et al. 2004) and acts by breaking ROS-induced chain reactions during lipid peroxidation. The most biologically active form of vitamin E is alpha-tocopherol, which inhibits oxidative stress-induced damage to sperm cell membranes (Mora-Esteves and Shin 2013).

In a double-blind, randomized, placebo crossover controlled trial, healthy men with high seminal ROS levels and normal female partners were given 600 mg/day vitamin E or placebo for 3 months. Serum levels of vitamin E increased after treatment, and zona-binding assay results improved, indicating that the treatment enhanced in vitro sperm function (Kessopoulou et al. 1995). In a prospective, randomized, placebo-controlled trial combining antioxidant and antiestrogen therapy, infertile men with idiopathic oligozoospermia were treated with a combination of vitamin E (400 mg/day) and clomiphene citrate (25 mg/day) for 6 months. In comparison to the placebo group, infertile men receiving the combination treatment had improved sperm count and progressive motility, while their partners showed a higher incidence of pregnancy (Ghanem et al. 2010). Oral supplementation of vitamin E (100 mg, 3 times/day) for 6 months in asthenozoospermic patients enhances sperm motility and leads to a reduction in lipid peroxidation in sperm cells as evidenced by less MDA concentrations. After supplementation, the female partners of approximately 20% of the patients receiving this oral therapy were able to achieve a pregnancy (Suleiman et al. 1996). After 1 month of treatment of fertile normozoospermic with vitamin E (200 mg/day), MDA levels were normalized, most likely due to a reduction of lipid peroxidation, and their fertilization rates per cycle increased, but no further changes were seen upon completion at 3 months (Geva et al. 1996). However, at a lower dose (100 mg × thrice daily) for 120 days, vitamin E given to idiopathic oligospermic men did not improve sperm parameters (Giovenco et al. 1987).

Eskenazi's study looked into the dietary and supplement intake of healthy, nonsmokers using a self-administered questionnaire. They found that men who had a higher antioxidant intake had increased sperm count and motility. Moreover, vitamin E intake was positively associated with higher motility, vitamin C was positively associated with higher sperm count, and beta-carotene intake was positively associated with both sperm count and motility (Eskenazi et al. 2005). Vitamin E supplementation (400 IU/day) of women undergoing controlled ovarian stimulation followed by intrauterine insemination (IUI) increased endometrial thickness, but implantation and pregnancy rates in these women were not higher than those in women who did not take vitamin E (Cicek et al. (2012).

In both normozoospermic and asthenozoospermic semen samples, adding 5 mM of vitamin E to the cryoprotective media prior to the freeze-thaw procedure caused a significant improvement in post-thaw motility and DNA integrity (Kalthur et al.

2011). These results are similar to those in a prior study in which the addition of vitamin E (either at 100 or 200 mM) to the cryopreservation medium improved the post-thaw motility of spermatozoa cryopreserved from both men with normal sperm parameters and men with abnormal sperm parameters (Taylor et al. 2009). In a study using teratozoospermic samples and sperm prepared by swim up and incubated with 40 μM alpha-tocopherol in media for 1 h, alpha-tocopherol was found to enhance sperm motility and viability in the teratozoospermic samples (Keshtgar et al. 2012).

Men taking vitamin E (400 mg) and selenium (225 μg) for 3 months had lower MDA levels and higher sperm motility than the men given vitamin B (4.5 g/day) for the same duration, indicating that the combination of vitamin E and selenium improved sperm quality (Keskes-Ammar et al. 2003). Infertile men with idiopathic asthenozoospermia who received oral supplementation of vitamin E (400 IU) and selenium (200 μg) for a minimum of 100 days showed improvement in either sperm motility, sperm morphology, or both, and about 11% reported spontaneous pregnancy in their partners. The investigators suggested that combined oral therapy of vitamin E and selenium was effective in treating men with idiopathic infertility who were asthenozoospermic or asthenoteratozoospermic (Moslemi and Tavanbakhsh 2011).

6.4.2 Vitamin C

Vitamin C (L-ascorbic acid or ascorbate) is a water-soluble antioxidant, which acts as a reducing agent for transition metal ions and free radicals (Buettner et al. 1991). Vitamin C plays an important role in the conversion of cholesterol to bile acids and tryptophan to serotonin, as well as in the activation of the B vitamin and folic acid (Chambial et al. 2013). The ascorbic acid content in seminal plasma and epididymal fluid is higher than that of blood plasma (Thiele et al. 1995; Smith et al. 1996). As a naturally occurring free radical scavenger, high ascorbic acid levels in seminal plasma protect sperm cells from oxidative stress and maintain DNA integrity (Dawson et al. 1987; Fraga et al. 1991). Men with leukocytospermia and men with a high percentage of sperm DNA damage have low seminal ascorbic acid levels (Song et al. 2006). Infertile men have lower seminal ascorbic acid levels than fertile men, and ascorbic acid is positively correlated with percentage of sperm with normal morphology (Colagar and Marzony 2009).

In oligozoospermic subfertile men, plasma and seminal ascorbic acid levels were lower than those of a control group (Ebesunun et al. 2004). However, oral supplementation of vitamin C (1000 mg × twice daily) for 2 months in oligozoospermic infertile men increased sperm count, sperm motility, and sperm morphology (Akmal et al. 2006). Adding 10 mM of ascorbic acid to semen samples before mixing them with cryomedia reduced cryopreservation-induced DNA damage in sperm from infertile men but not in sperm from fertile men. However, vitamin C supplementation was unable to overcome the detrimental effects of cryopreservation on sperm

parameters, as there were no improvements seen in post-thaw sperm count, motility, and morphology compared to the controls (Branco et al. 2010). Ascorbic acid addition (600 μM) in vitro (1 h incubation time) could protect teratozoospermic samples from the effects of oxidative stress, as evidenced by lower levels of sperm MDA and DNA damage and high percentages of sperm progressive motility and viability (Fanaei et al. 2014).

The percentage of sperm DNA fragmentation in men taking vitamin C (1 g) and vitamin E (1 g) daily for 2 months was markedly lower than in sperm of men taking placebo (Greco et al. 2005a). Vitamin C may act synergistically with vitamin E to improve sperm quality (Ko and Sabanegh 2014). Infertile men with an elevated percentage of sperm DNA fragmentation whose partners had failed one ICSI attempt were given vitamin C (1 g) and vitamin E (1 g) daily for 2 months. In this study, 76% of the men showed a decrease in sperm DNA fragmentation, and upon a second ICSI attempt, their partners had higher clinical pregnancy rates and implantation rates compared to their first ICSI attempt (Greco et al. 2005b). However, in a double-blind, placebo-controlled study, sperm parameters were unchanged in infertile men with asthenozoospermia or oligozoospermia given vitamin C (1000 mg) and vitamin E (800 mg) for 56 days (Rolf et al. 1999).

Smoking infertile men had lower seminal ascorbic acid levels than non-smoking infertile men, while the fertile men (regardless of smoking status) had higher seminal ascorbic acid levels than the infertile men (Mostafa et al. 2006). Seminal ascorbic acid levels in men (smokers or not) were correlated positively with sperm concentration and motility and negatively with abnormal sperm morphology (Mostafa et al. 2006). In a prospective study in women undergoing in vitro fertilization and embryo transfer (IVF/ET) (n = 76, of which 38 were smokers), vitamin C (500 mg/day) slow release supplementation was given to 19 women who smoked and 19 women who were non-smokers. Women with vitamin C supplementation had higher ascorbic acid levels in their follicles than the controls. Pregnancy rates in the Vitamin C-supplemented women were higher among the non-smokers than in the smokers (Crha et al. 2003).

6.4.3 Folate

Folate is a naturally occurring form of the vitamin B9 that is found in food. Folate inhibits lipid peroxidation via its antioxidant properties (Joshi et al. 2001). Folate, a methyl donor, is an essential substrate in DNA synthesis and in RNA precursor synthesis. It is also essential for the remethylation of homocysteine to methionine. Cobalamin (vitamin B12) is the cofactor to methionine synthase in folate-dependent homocysteine remethylation. Cobalamin deficiency can impair DNA synthesis and elevate blood total homocysteine levels (Varela-Moreiras et al. 2009). It is important to underline that oligozoospermic men have lower cobalamin levels than normozoospermic men (Tomaszewski et al. 1963). In addition, infertile men have lower serum folate levels than fertile men (Murphy et al. 2011). Men with azoospermia have

lower seminal homocysteine and cobalamin but not folate levels than normozoospermic men. However, men with obstructive azoospermia have higher folate and cobalamin levels than men with nonobstructive azoospermia (Crha et al. 2010).

In a double-blind, placebo-controlled intervention study consisting of infertile men and fertile men, subjects were given folic acid (5 mg), zinc sulfate (66 mg), folic acid and zinc sulfate, or two placebos. Total normal sperm count increased after combined treatment of folic acid and zinc sulfate in both the infertile and fertile men (Wong et al. 2002). In more recent double-blind, placebo-controlled intervention study, fertile men and infertile men were given daily treatment of folic acid (5 mg/day) and zinc sulfate (66 mg/day) or placebo for 26 weeks. At the end of the treatment period, the infertile men taking folic acid and zinc sulfate had a higher sperm concentration, although the mechanism behind the increase was not likely due to changes in testosterone, follicle-stimulating hormone (FSH), or inhibin B levels (Ebisch et al. 2006).

A randomized trial was conducted to assess the effects of low-dose folic acid (0.4 mg/day) use on the ovarian response to mild and conventional stimulation among infertile women (Twigt et al. 2011). The women taking low-dose folic acid had reduced follicular response to conventional stimulation, regardless of preovulatory follicular count or serum anti-Mullerian hormone levels. Endocrine responses were also reduced. These findings indicate that folic acid exerts its effects mainly during early follicular development. Women undergoing IVF/ET and given folic acid had lower follicular fluid and serum levels of homocysteine. These women also produced better quality oocytes and with higher oocyte maturity than the women who did not receive folic acid (Szymanski and Kazdepka-Zieminska 2003). Women on folic acid supplementation have lower homocysteine concentrations in follicular fluid (Boxmeer et al. 2008). The quality of the embryo is lower when homocysteine concentrations in follicular fluid are high (Ebisch et al. 2006), which is likely due to follicular oxidative stress. As such, follicular fluid homocysteine levels could be an indicator of a women's pregnancy potential, whereby lower homocysteine levels in follicular fluid are correlated to higher chances of a clinical pregnancy (Ocal et al. 2012).

Women with polycystic ovary syndrome (PCOS) have poor quality oocytes and embryos along with low fertilization rates. In a study of women with PCOS who were seeking assisted reproduction, follicular fluid homocysteine levels were negatively associated with follicular fluid folate, vitamin B12 levels, and fertilization rates but were positively correlated with follicular fluid MDA levels. Women with higher follicular fluid homocysteine levels had lower follicular fluid vitamin B12 levels, and these women also had higher numbers of Grade 3 embryos compared to Grade 1 and 2 embryos (Berker et al. 2009). Myoinositol is a molecule that belongs to the vitamin B family. Myoinositol enhances the developmental competence of maturing oocytes (Goud et al. 1999). In a prospective-controlled randomized trial, the effects of myoinositol among women with PCOS who were scheduled for ovulation induction and ICSI were examined. Myoinositol improves ovulatory function and aids in oocyte maturation, increasing oocyte quality and the number of oocytes collected after ovarian stimulation in IVF patients. Women

co-treated with 2 g myoinositol and 400 μg folic acid had lower numbers of germinal vesicles and degenerated oocytes at ovum pickup without affecting the total number of retrieved oocytes (Papaleo et al. 2009). Women co-treated with 2 g myoinositol and 200 μg folic acid had lower numbers of immature oocytes at ovum pickup; however, in this study, the women treated with myoinositol had a greater number of oocytes recovered during pickup (Ciotta et al. 2011). These investigators suggested that a combination of myoinositol and folic acid may be helpful in treating PCOS patients.

6.4.4 Coenzyme Q10

Coenzyme Q10 (CoQ10) is a lipid-soluble element present as part of the respiratory chain in mitochondria. CoQ10 can be present in the reduced form, ubiquinol, which acts as a potent antioxidant, and in the oxidized form, ubiquinone (Lanzafame et al. 2009). Oral supplementation of CoQ10 for 6 months in infertile men with idiopathic asthenozoospermia increased levels of CoQ10 in seminal plasma and sperm cells and also increased sperm motility (Balercia et al. 2004). CoQ10 concentrations in the seminal plasma are directly correlated with sperm count and motility (Lanzafame et al. 2009). In a randomized placebo-controlled trial, 60 infertile men with idiopathic oligoasthenoteratozoospermia (OAT) were given 200 mg/day of CoQ10 or placebo for 3 months (Nadjarzadeh et al. 2014). Upon completion, CoQ10 levels increased in the supplemented group. There was also a positive correlation between seminal CoQ10 levels, CAT and SOD levels, as well as normal sperm morphology, suggesting that intake of CoQ10 could help decrease oxidative stress in OAT infertile men (Nadjarzadeh et al. 2014). However, in an earlier study by the same group, supplementation with CoQ10 did not lead to improvement in sperm parameters. In that double-blind, placebo-controlled clinical trial, 47 infertile men with idiopathic OAT were randomized to receive either 200 mg CoQ10 or placebo for 12 weeks. Although the men taking the supplement had higher seminal total antioxidant capacity as well as lower levels of lipid peroxidation, their sperm quality showed no significant improvement (Nadjarzadeh et al. 2011).

In a double-blind, placebo-controlled randomized study also done in men with idiopathic OAT (n = 228), 114 infertile men were given oral supplementation of 200 mg ubiquinol for 26 weeks (Safarinejad et al. 2012). Sperm count, motility, and normal morphology all had increased by the end of this period. After a 12-week washout period, sperm count and motility remained increased, while normal sperm morphology returned to baseline values (Safarinejad et al. 2012). In a prior study on 212 men with idiopathic OAT, patients (n = 106) received 300 mg/day CoQ10 for 26 weeks followed by a 30-week washout phase (Safarinejad 2009). This study showed that treatment with CoQ10 was positively correlated with sperm density, motility, and morphology. In the author's follow-up study, the effects of CoQ10 supplementation on pregnancy rates were assessed using 287

infertile men with idiopathic OAT who were treated per oral with CoQ10 (300 mg × twice daily) for 12 months (Safarinejad 2012). After a year of CoQ10 supplementation, the treated men showed improvement in sperm quality and pregnancy rates improved in their partners. A systematic review and meta-analysis on male infertility patients treated with CoQ10 (149 males and 147 controls) found that CoQ10 therapy increased seminal coenzyme Q10 levels, sperm concentration, and motility; however, CoQ10 supplementation did not increase pregnancy rates (Lafuente et al. 2013).

6.4.5 Trace Elements

Zinc and selenium concentrations are positively correlated in the seminal plasma (Xu et al. 1993). Unlike in oligozoospermic men, higher selenium and zinc levels were positively correlated with sperm density in normozoospermic men (Xu et al. 1993). Treating infertile men with idiopathic asthenozoospermia and/or oligozoospermia orally with zinc sulfate increased seminal zinc levels and led to an improvement in sperm progressive and total motility (Kynaston et al. 1988). Similarly, infertile men with idiopathic oligozoospermia who were given zinc sulfate (200 mg) for 4 months showed increased levels of seminal zinc (but not serum zinc), increased sperm count and motility, and sperm with normal morphology (Tikkiwal et al. 1987). Another study found that men with oligoasthenozoospermia had higher zinc levels and poorer sperm membrane integrity than men with normozoospermia, suggesting that the uptake of excess zinc into the sperm impairs membrane integrity and lowers motility (Carpino et al. 1998). Seminal zinc levels in fertile men (regardless of smoking status) were higher than those in infertile men, whereas smokers had lower seminal zinc levels than non-smokers (Colagar et al. 2009). Animal studies suggest that zinc is an essential trace element that plays a role in maintaining germ cells and in the continuation of the spermatogenesis process. Zinc plays important in the regulation of sperm motility (Yamaguchi et al. 2009).

Selenium is involved in the development of the testis and ovaries and in gametogenesis and fertilization. Selenium supplementation at doses <200 μg/day was found to enhance male fertility (Mirone et al. 2013). In a double-blind, placebo-controlled randomized study on 468 infertile men with idiopathic oligoasthenoteratozoospermia, each treatment group received daily oral supplementation of either selenium (200 μg), *N*-acetyl-cysteine (NAC, 600 mg), selenium (200 μg) + NAC (600 mg), or placebo for 26 weeks followed by a 30-week treatment-free duration (Safarinejad and Safarinejad 2009). In this study, selenium and NAC supplementation improved sperm concentration, motility, and normal morphology (Safarinejad and Safarinejad 2009). Oral intake of selenium seems to have a positive effect on pregnancy outcomes (Mirone et al. 2013). Recent study suggests that oral supplementation with selenium may reduce the incidence of preeclampsia in pregnant women with low selenium levels (Rayman et al. 2014).

6.4.6 *Lycopene*

Lycopene, a potent $^1O_2$ quencher, belongs to the carotenoid family. It acts on H_2O_2 and also halts the propagative chain reactions (Rao and Agarwal 1999). Oral supplementation of lycopene (2 mg × twice daily) for 3 months in infertile men with idiopathic nonobstructive oligoasthenoteratozoospermia improved sperm concentration, motility, and morphology (Gupta and Kumar 2002). In another study, 50 infertile men were given 8 mg of lycopene daily, which lead to an improvement in all sperm parameters (Mohanty et al. 2001). Studies involving lycopene supplementation generally show reduced lipid peroxidation and DNA damage with better sperm count and viability. However, the role of lycopene in treating idiopathic male infertility requires further extensive clinical research (Durairajanayagam et al. 2014).

6.5 Effect of the Combination of Antioxidants on Fertility

In view of the adverse effects of oxidative stress on gamete quality, fertilization rates, and pregnancy outcome, intake of antioxidant mixture with different antioxidant activities would provide greater antioxidant protection than that of one antioxidant and might be useful for treatment of human infertility (Comhairea and Decleer 2012). Table 6.2 provides an overview of various antioxidants, their location in vivo, and mechanism of action. The use of multiple antioxidants, each with a different mode of action along with an anti-inflammatory substance that quells leukocyte-derived ROS, is more beneficial in improving sperm quality and pregnancy rates than monotherapy (Tremellen 2008). The effect of combination antioxidant therapy was tested in a prospective randomized, double-blind, placebo-controlled trial in which couples with severe male factor infertility were given for 3 months a capsule/day of Menevit, which is a combination of vitamin C (100 mg), vitamin E (400 IU), folate (500 μg), lycopene (6 mg), zinc (25 mg), selenium (26 μg), and garlic (1000 mg). Women whose partners were supplemented with Menevit prior to their IVF cycle had higher pregnancy rates, although embryo quality and oocyte fertilization rates were similar to those in the control group (Tremellen et al. 2007). In another study, 58 men with sperm DNA damage (sperm DNA fragmentation index >15%) who had at least two prior unsuccessful attempts at IVF/ICSI were given daily oral antioxidant therapy consisting of vitamin C (400 mg), vitamin E (400 mg), beta-carotene (18 mg), zinc (500 μmol), and selenium (1 μmol) for 13 weeks. Sperm DNA fragmentation index improved following such antioxidant therapy. However, sperm decondensation also increased following treatment, which could negatively affect ART outcomes (Menezo et al. 2007).

Table 6.2 Selected antioxidants and their solubility, location in vivo, and mechanism of action

Antioxidant	Solubility	Location in vivo	Mechanism of action
Catalase	–	Cellular peroxisome	Decomposes hydrogen peroxide to molecular oxygen and water
Superoxide dismutase	–	Cytoplasm, mitochondria, extracellular	Dismutation of superoxide anion to hydrogen peroxide
Glutathione peroxidase	–	Cytosol, mitochondrial matrix	Reduces hydrogen peroxide to water, oxidizes glutathione to glutathione disulfide
Vitamin E	Lipid	Interior cell membranes	Directly scavenges superoxide anion, upregulates antioxidant enzymes, inhibits lipid peroxidation, interrupts chain-breaking propagative reaction
Vitamin C	Water	Cytosol	Scavenges superoxide anion, interrupts chain-breaking propagative reaction, acts synergistically with vitamin E
Folate (Vitamin B_9)	Water	Not synthesized de novo	Methyl donor → remethylation of homocysteine to methionine
Melatonin	Lipid	Crosses all membranes → reaches mitochondria, nuclei	Directly scavenges hydroxyl radical and other free radicals, activates superoxide dismutase, catalase
Coenzyme Q_{10}	Lipid	All cell membranes (inner mitochondrial membrane as ubiquinone and extra mitochondrial membrane as ubiquinol)	Inhibits lipid peroxidation (interrupts chain-breaking propagative reaction), reduces mitochondrial oxidative stress, ubiquinol acts in concert with alpha-tocopherol
L-carnitine	Lipid	Cell membranes	Mitochondrial oxidation of long-chain fatty acids (source of cellular energy supply), neutralizes free radicals, scavenges superoxide anion
Zinc	–	Organs, tissues, body fluids, SOD of cytosol, and extracellular space	Produces other antioxidant molecules, reduces hydroxyl radical formation from hydrogen peroxide
Selenium	–	Cytosol	Component of antioxidant enzymes—acts through glutathione peroxidase, acts synergistically with vitamin E
Lycopene	Lipid	Cell membranes	Quenches singlet oxygen, acts on hydrogen peroxide, interrupts chain-breaking propagative reaction

6.6 Conclusions

ROS-induced oxidative stress is an important contributing factor to negative ART outcomes. As such, it is important that ROS is minimized at every step possible during the IVF/ICSI procedure. The use of antioxidants in vivo and in vitro could be employed as a strategy of minimizing oxidative stress during IVF/ICSI procedures. For example, culture media could be supplemented with antioxidants such as vitamins C and E to benefit the developing embryo. Adding ascorbate to cryopreservation media could help preserve sperm motility and viability following freeze-thaw procedures. Oral intake of antioxidants to enhance reproductive function in couples with poor gamete quality has been investigated via observational studies as well as randomized controlled studies. However, data currently available on antioxidant use as an oral supplementation for improving ART outcomes in couples planning for IVF/ICSI cycles remains inconclusive. A Cochrane review of randomized controlled trials on oral antioxidant supplementation for male partners of couples seeking ART (34 trials, 2876 couples) found that the female partners of men who took oral antioxidants had higher pregnancy rates and life birth rates than those of the controls (Showell et al. 2011). Another Cochrane review of randomized controlled trials on oral antioxidant supplementation, this time in women attending reproductive clinics (28 trials, 3548 women), showed very low quality of evidence and that there was no association between antioxidant intake and clinical pregnancy rates or life birth rates in these women (Showell et al. 2013). Based on individual studies, some antioxidants do show promising results. However, further larger, well-designed studies in infertile couples are required to strengthen the study findings.

The etiology of infertility and the cause for high ROS levels in each couple is variable, so antioxidant supplementation could be administered once the underlying cause of infertility/high ROS levels is determined or if no particular etiology can be determined (Cocuzza and Agarwal 2007). However, the appropriate type and dosage of antioxidant(s) need to be determined, as the goal remains to minimize excessive ROS formation and complement the endogenous antioxidant levels in the gamete and reproductive tract. Although the formation of ROS during IVF cannot be avoided, it is important to be aware of the possible routes of excessive ROS generation in order to preempt its increased formation as much as possible. Good quality research is needed in the area of antioxidant supplementation either on its own or in combination, which specifically enriches the gonads and reproductive tract. At the same time, further research is also required in the ART field so as to ameliorate protocols with the goal of providing the gametes/developing embryo with conditions that mimic in vivo conditions as closely as possible.

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Chapter 7
Plant Antioxidants in the Prevention of Early Life Programming Diseases

Kaïs Hussain Al-Gubory

Abstract An unavoidable consequence of aerobic life is the production of reactive oxygen species (ROS), which can damage proteins, fatty acids and nucleotides that make up our cells and lead to organ dysfunction and diseases. Aerobic organisms elicit interrelated antioxidant mechanisms to keep ROS at physiological levels and to ensure cellular redox homeostasis and biological processes during the life cycle of organisms, namely, cell proliferation, differentiation, migration, survival and apoptosis. The delicate balance between ROS production and removal by antioxidants during pregnancy is crucial for the foetus to develop and mature into a healthy neonate, whereas early foetal life ROS-induced oxidative stress alters foetal developmental trajectory that could lead to increased risk of noncommunicable chronic diseases during adult life. Stressful events during the intrauterine life, such as undernutrition, malnutrition, unhealthy lifestyle behaviours and exposure to multiple human-made pollutants, adversely affect prenatal development and may contribute to foetal origin of disease in adulthood partly due to oxidative stress. Antioxidants present in most foods of vegetable origin are widely recommended for health promotion and disease prevention. In this chapter, the risk of adverse prenatal development related to antioxidant deficiencies and the importance of dietary antioxidants in the establishment of healthy pregnancy are highlighted. The potential of medicinal plants and plant-based functional foods and beverages with varied and balanced antioxidants, as well as diet quality and healthy dietary habits, as effective means in the prevention of oxidative stress and prenatal developmental disorders is also discussed.

Keywords Pregnancy • Environmental factors • Reactive oxygen species • Oxidative stress • Foetal programming of adult diseases • Phytonutrients • Antioxidant therapy

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7.1 Introduction

Aerobic organisms need oxygen for nutrient oxidation and energy production by healthy mitochondria in the form of adenosine triphosphate (ATP), and they paradoxically must control the generation of unavoidable reactive oxygen derivatives (Davies 1995), which are collectively referred to as reactive oxygen species (ROS). Under these conditions, aerobic organisms elicit interrelated antioxidant mechanisms to keep ROS at physiological levels (Fridovich 1998) and to ensure cellular redox homeostasis and developmental processes during the life cycle of organisms, namely, cell proliferation, differentiation, migration, survival and apoptosis (Veal and Day 2011; Chaudhari et al. 2014; Redza-Dutordoir and Averill-Bates 2016). Oxidative stress is a state of an imbalance between the production of ROS and the ability of the cell to counteract their harmful oxidative effects through neutralization by enzymatic and nonenzymatic antioxidants. An excessive ROS production or a deficiency of antioxidants damages proteins, fatty acids and nucleotides and disturbs mitochondrial function and signalling events (Ježek and Hlavatá 2005) that lead to organ dysfunction and increase susceptibility to a range of metabolic disorders and noncommunicable diseases (NCDs), such as adiposity, type II diabetes, nephropathy, atherosclerosis, hypertension, cardiomyopathy, cardiac hypertrophy, congestive heart failure, rheumatoid arthritis, cancer and neurodegeneration (Cui et al. 2004; Pieczenik and Neustadt 2007; Valko et al. 2007; Mole and Ratcliffe 2008; Pham-Huy et al. 2008; Golbidi and Laher 2010; Sims and Muyderman 2010; Rahman et al. 2012; Ray et al. 2012; Subramaniam and Chesselet 2013; Camps and García-Heredia 2014; Montezano and Touyz 2014; Görlach et al. 2015).

Adverse events during the intrauterine life, such as undernutrition, malnutrition, oxidative stress and foetal hypoxia, can potentially induce prenatal development complications and disorders that contribute to foetal origin of disease in adulthood (Fernandez-Twinn and Ozanne 2006; Luo et al. 2006; Ghulmiyyah et al. 2011; Lakshmy 2013; Vuguin et al. 2013; Giussani et al. 2014; Ávila et al. 2015; Kimani-Murage et al. 2015; Saad et al. 2016). This is consistent with the hypothesis of early foetal origins of adult disease or the concept of early programming of adult diseases (Barker et al. 1993). It has been suggested that adaptation of the early developing foetus to maternal undernutrition increases the risk of NCDs in adult life (Langley-Evans 2015). Of major concern are also unhealthy lifestyle behaviours, such as tobacco smoking, alcohol consumption and medical drug abuse, as well as maternal exposure to various environmental pollutants, including heavy metals, pesticides, endocrine disruptors and ambient air particulate matter, which can produce developmental toxicity and increase the susceptibility of offspring to development complications (Wells et al. 2009; Luo et al. 2010; Backes et al. 2013; Erickson and Arbour 2014; Ding et al. 2015). Increased ROS production induced by cumulative exposures to multiple environmental factors during prenatal development (Al-Gubory 2014a) has been suggested to be an underlying mechanism of developmental toxicity and increased disease risk in adulthood (Al-Gubory 2014b). In this chapter, the risk of adverse prenatal development related to antioxidant deficiencies and the importance of dietary antioxidants in the establishment of healthy pregnancy are highlighted. The potential of medicinal plants, plant-based functional foods and beverages with varied and balanced antioxidants, as well as diet quality and healthy

dietary habits as effective means in prevention of oxidative stress and prenatal developmental disorders, is also discussed.

7.2 Reactive Oxygen Species, Oxidative Stress and Prenatal Development

The development of conceptus (embryo/foetus and extraembryonic/foetal membranes) and its attachment and interaction with the epithelial tissue lining the inner cavity of the uterus ensure the establishment of placenta, which transfers nutrients and oxygen to the early developing embryo/foetus and contributes to a healthy pregnancy (Gude et al. 2004). Low oxygen availability to the early developing conceptus is essential for appropriate embryonic development before the establishment of placental tissues (Caniggia et al. 2000; Simon and Keith 2008). Then the increasing placental and foetal demands for oxygen are required for sustaining high mitochondrial oxidative metabolism and energy production (Murray 2012). Therefore, the early developing embryo and foetus have to deal with an environment where oxygen homeostasis is largely disturbed.

Cells and tissues within organs of biological systems respond to changes in oxygen levels via a pathway involving a specific oxygen sensor (Ratcliffe et al. 1998). Hypoxia-inducible factors (HIFs) discovered in the early 1990s (Semenza and Wang 1992) are key transcriptional regulators of the mammalian response to hypoxia (Majmundar et al. 2010). Under conditions of hypoxia, HIFs function as activators of vascular endothelial growth factor gene transcription (Levy et al. 1995; Forsythe et al. 1996) and thereby play a crucial role in the regulation of embryonic cardiovascular development and physiology (Semenza et al. 1999). Nitric oxide synthase (NOS)-$NO^{\bullet}$ pathway is an important element in the regulation of HIFs because upregulation of HIFs by $NO^{\bullet}$ is an important mechanism by which $NO^{\bullet}$ modulates responses to hypoxia in mammalian cells (Agani et al. 2002). Importantly, hypoxia enhances NOS expression in the ovine foetal adrenal (Monau et al. 2009) and guinea pig foetal heart (Thompson et al. 2009a).

Barcroft's Everest in utero hypothesis stemming from studies carried out in sheep postulated that the foetus develops in an environment comparable to that endured by an adult on the summit of Mount Everest (Barcroft 1946; Eastman 1954). Under conditions of in utero hypoxia, oxygen sensing is central for early embryonic and foetal developmental processes. Despite early pregnancy hypoxia, the control of ROS generation and maintenance of their concentrations at physiological levels appear to be one of the central elements in the mechanisms of cellular signal transduction pathways involved in cell proliferation and gene regulation, differentiation and development (Janssen-Heininger et al. 2008). Conditions of in utero development where the foetus is exposed to high levels of ROS that exceed its antioxidant capacity lead to oxidative stress, alter cell function and integrity and ultimately result to adverse prenatal development (Al-Gubory et al. 2010; Dennery 2010). Therefore, ROS generated by a variety of intrauterine conditions play important roles in numerous developmental processes when they are produced at the right levels, and they may exert damaging effects when they are overabundant (Fig. 7.1).

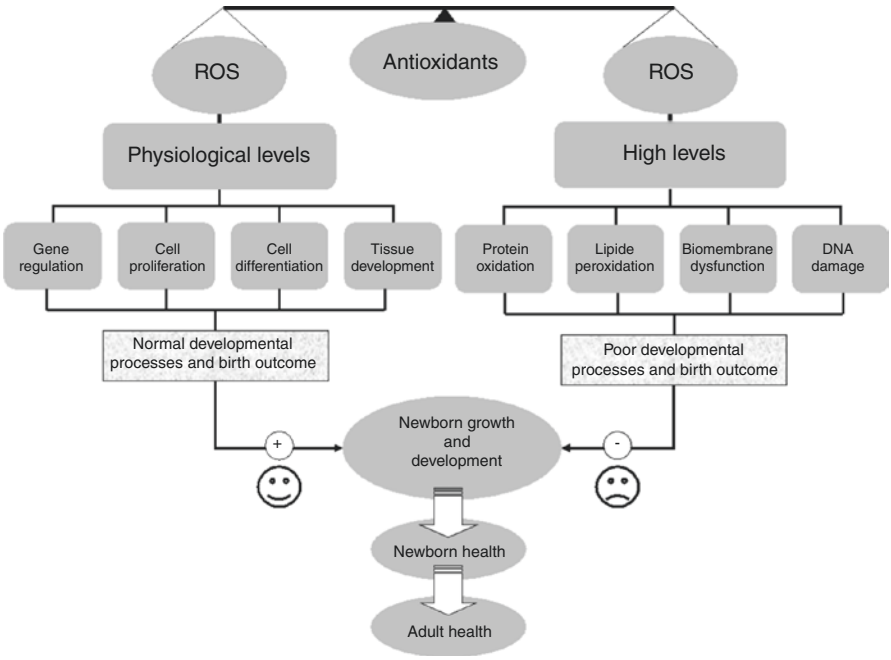

Fig. 7.1 Physiological level of reactive oxygen species (ROS) plays important roles in normal developmental processes that positively impact newborn growth, development and health through the regulation of gene expression, cell proliferation and differentiation and tissue development. Adversely, high levels of ROS damage cellular macromolecules and ultimately lead to poor developmental processes and birth outcome that negatively impact newborn growth, development and health outcome

Abnormal development of the placental terminal villous tree (Krebs et al. 1996) and placental oxidative stress (Myatt et al. 1996; Wang and Walsh 2001; Myatt and Cui 2004; Poston and Raijmakers 2004) are major factors involved in a number of human pregnancy disorders and pathologies of placental origin including foetal intrauterine growth restriction (IUGR) and preeclampsia (Myatt 2006). Under conditions of IUGR, blood levels of the lipid peroxidation products, F2-isoprostanes (F2-IsoPs) and malondialdehyde (MDA), are elevated (Hracsko et al. 2008). Redox imbalance, cell dysfunction and reduced protein synthesis are the major mechanisms responsible for abnormal absorption and metabolism of nutrients and impaired development of the small intestine, liver and muscle of pig neonates with IUGR (Wang et al. 2008). It is important to note that chronic inhibition of $NO^{\bullet}$ synthesis in pregnant rats leads to sustained hypertension, proteinuria, thrombocytopenia and IUGR (Molnár et al. 1994). Dietary L-arginine prevents IUGR in rats suggesting an important role of $NO^{\bullet}$/L-arginine signalling pathway for foetal development (Vosatka et al. 1998). IUGR is associated with increase of $NO^{\bullet}$ production in umbilical venous plasma (Lyall et al. 1996) and placenta (Tikvica et al. 2008). A decline in oxygen and nutrient supply to the foetus and oxidative stress-induced

trophoblast cell death are among the identified disorders associated with IUGR in humans (Scifres and Nelson 2009). Human and animal studies indicate that oxidative stress increases with IUGR. Therefore, the use of antioxidants to combat oxidative stress-related complications may be useful in the prevention or treatment of IUGR (Table 7.1), which is still a major cause of perinatal morbidity and mortality in underdeveloped and developing countries (Sharma et al. 2016).

Table 7.1 Oxidative stress and biomarkers of intrauterine growth restriction (IUGR)

Results	Biological samples	Biomarkers	Species	Reference
Decreased uptake of glucose and ATP production	Skeletal muscle	Adenosine triphosphate	Rat	Selak et al. (2003)
Increased lipid peroxidation and decreased antioxidant enzyme activities	Neonates cord blood	Malondialdehyde, superoxide dismutase Catalase Glutathione	Human	Gupta et al. (2004)
Increased lipid peroxidation and protein oxidation	Maternal and foetal erythrocyte	Malondialdehyde	Human	Kamath et al. (2006)
Increased lipid peroxidation	Maternal and umbilical cord plasma	Malondialdehyde	Human	Biri et al. (2007)
Increased lipid peroxidation and decreased total antioxidant capacity	Serum	Malondialdehyde 4-hydroxyalkenals	Human	Karowicz-Bilinska et al. (2007)
Increased lipid peroxidation and decreased antioxidant enzyme activities	Neonates cord blood	Malondialdehyde Superoxide dismutase Glutathione peroxidase	Human	Hracsko et al. (2008)
Increased offspring susceptibility to high-fat diet-induced mitochondrial dysfunction	Skeletal muscle		Pig	Wang et al. (2008)
Increased DNA oxidation	Urine	8-Oxo-7,8-dihydro-2′-deoxyguanosine	Human	Potdar et al. (2009)
Decreased levels of proteins regulating oxidative defence, immune function, and tissue growth	Jejunum, liver muscles	Peroxiredoxin 1 zeta-crystallin Translation initiation factor-3 Beta-actin Transferrin Eukaryotic desmin Keratin 10	Pig	Liu et al. (2012)
Increased DNA damage and lipid peroxidation	Umbilical, cord serum	Malondialdehyde, 8-hydroxy-2-deoxyguanosine	Human	Negi et al. (2012)

7.3 Environmental Factors, Oxidative Stress and Foetal Programming of Adult Diseases

Undernutrition, malnutrition, unhealthy lifestyle behaviours and exposures to environmental pollutants affect prenatal development and may contribute to foetal origin of disease in adulthood partly due to oxidative stress (Fig. 7.2). In utero ROS-induced oxidative stress alters foetal developmental trajectory that could lead to increased risk of NCDs during adult life (Luo et al. 2006). Mitochondria are sensors of environmental factors and initial mediator of oxidative stress, organ dysfunction and foetal programming of adult diseases (Leduc et al. 2010; Simmons 2012; Stangenberg et al. 2015). Additionally, mitochondria are the first targets for ROS-induced oxidative damage and mitochondrial dysfunction; both can be induced by exposure to many environmental pollutants (Franco et al. 2007a; Chang et al. 2013; Caito and Aschner 2015; Li et al. 2015; Lee and Yu 2016). Of note is that foetal adaptive responses to environmental factors are mediated by epigenetic

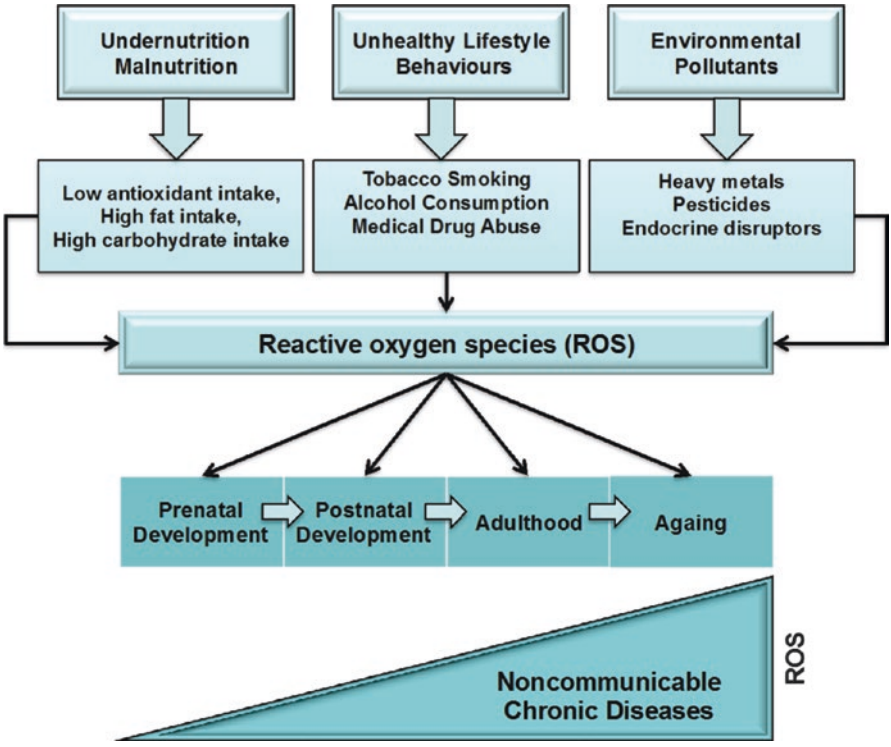

Fig. 7.2 Undernutrition, malnutrition, unhealthy lifestyle behaviours and multiple exposures to environmental pollutants adversely affect prenatal development and may contribute to foetal origin of disease in adulthood due to reactive oxygen species (ROS)-induced oxidative stress

changes in the genome that may be generated by oxidative stress (Ávila et al. 2015).

A number of environmental stressors including lack of safe drinking water, poor sanitation and air quality, persistent inorganic or organic compounds and poor quality of women's diets (Torheim et al. 2010) are predicted to increase with climate change and increasingly affecting the health of pregnant women and children (Rylander et al. 2013; Poursafa et al. 2015). Given the fast-changing environment all around the world, prenatal maternal undernutrition and malnutrition (Jiang et al. 2015; Kimani-Murage et al. 2015; Mekonen et al. 2015; Rachmi et al. 2016; Sarki et al. 2016; Jaacks et al. 2017), unhealthy lifestyle behaviours and increasing exposure to human-made pollutants (Ding et al. 2015; Vieira 2015; Bloom et al. 2016; Naksen et al. 2015; Xia et al. 2016) continue to be major concerns to pregnant women and their children in the developed, developing, underdeveloped and low-income countries.

7.3.1 Undernutrition/Malnutrition

The nutritional status of women before and during pregnancy determines maternal, foetal and offspring health. An increased risk of IUGR and perinatal mortality has been reported during the Dutch famine (Smith 1947). Mothers exposed during early pregnancy to this same famine (1944–1945) had offspring with birth weights lower than mothers not exposed to famine (Lumey 1992). Poor socio-economic conditions associated with undernutrition are likely to expose mothers to a high risk of IUGR (Neel and Alvarez 1991; Mavalankar et al. 1992; Spencer et al. 1999; Kramer et al. 2000). A birth weight below 2.5 kg reported by the World Health Organization (WHO) contributes to neonatal mortality and morbidity (WHO 1992), and affects more than 20 million infants in the world today, 95.6% of which are born in developing countries (WHO 2004). Maternal undernurition, placental insufficiency and impaired foetal usage of nutrients are the main causes of IUGR (Henriksen 1999) that predispose the infant to NCDs in adulthood (Ross and Beall 2008).

Animal studies have identified links between maternal undernutrition and foetal oxidative stress that can influence offspring health later in life. Offspring born from diet-restricted pregnant rats (50% of normal intake diets) exhibit oxidative stress characterized by increased superoxide radical $^{\bullet}O_2^-$ concentration and decreased the superoxide dismutase (SOD) (Franco Mdo et al. 2002). Offspring born from vitamin-restricted pregnant rats (50% vitamin-restricted diet) exhibit oxidative stress and have a high percentage of body fat and plasma triglycerides, suggesting that maternal dietary vitamin restriction predisposes the offspring to insulin resistance syndrome during adult life (Venu et al. 2004). Foetal nutrient insufficiency induced in pregnant sheep by reducing placental size and function increases offspring adiposity in early postnatal life (De Blasio et al. 2007). Lipid peroxidation in placenta evaluated by measuring Thiobarbituric Acid Reactive Substances' (TBARS) increases in prenatally malnourished rats with subsequent disturbance in kidney

proximal tubule sodium ATPases activity and renal dysfunction in offspring (Vieira-Filho et al. 2009). Maternal undernutrition increases ovarian oxidative stress in adult rat offspring (Bernal et al. 2010). In rats, malnutrition during pregnancy increased maternal hepatic and adult offspring renal MDA levels (Vieira-Filho et al. 2011).

Maternal malnutrition is associated with increased serum MDA and reduced activities of erythrocyte SOD and catalase (CAT) and level of reduced glutathione (GSH) in cord blood of the small for gestational age newborns (Gupta et al. 2004). Proteins provide amino acids for the synthesis of antioxidant enzymes, as well as those required for GSH and taurine synthesis. Animal and human studies demonstrate that adequate protein nutrition is crucial for the maintenance of cellular GSH levels and homeostasis (Wu et al. 2004). GSH is the most abundant intracellular low-molecular-weight tripeptide containing a thiol group, which is critical for the biological activity of GSH thiol. GSH is a major nonenzymatic antioxidant composed of the amino acids glutamate, cysteine and glycine. Cysteine is obtained from diet and synthesized from the methionine product, homocysteine, by transsulfuration, with cystathionine as an intermediate. L-cysteine plays a crucial role in cellular homeostasis as a precursor for synthesis of GSH and taurine within the liver (Stipanuk et al. 2006). Taurine is the most abundant free amino acid in the human placenta (Philipps et al. 1978) and plays a critical role in foetal growth and development (Sturman 1988). A low-protein diet (8% protein) and devoid of taurine increase the susceptibility of rat foetal pancreatic β-cells to damage induced by interleukin 1 (IL-1) and NO•, whereas taurine supplementation protects foetal islet cells against the cytotoxicity induced by IL-1 and NO• (Merezak et al. 2001).

Periconceptional undernutrition in sheep has been shown to affect maternal taurine homoeostasis and may ultimately alter offspring development (Thorstensen et al. 2012). Taurine supplementation (0.5 and 1 g/kg bodyweight/day) from gestational day 5 to gestational day 12 reduces embryo lethality and protects the developing embryos against maternal hyperglycaemia-induced oxidative stress in rats (Shivananjappa and Muralidhara 2012). Taurine transporter activity is reduced in human placentas collected during the first trimester of pregnancy (7–13 weeks) and at term (38–40 weeks) from women under conditions of obesity and preeclampsia compared to mothers of ideal weight and normal pregnancy (Desforges et al. 2013). These authors suggest that this reduction in taurine transporter activity can reduce placental taurine concentrations and taurine transfer to the foetus that may contribute to the increased risk of foetal growth restriction.

Maternal deficiencies of trace elements, mainly zinc, copper and manganese, have been implicated in infertility, pregnancy wastage, pregnancy-induced hypertension, placental abruption, premature rupture of membranes and low birth weight (Pathak and Kapil 2004). The imbalance between the ROS and the antioxidants associated with deficiencies in micronutrients impairs proper placental development and has persistent adverse effects on foetal development and neonatal tissues (Jansson and Powell 2007). Organogenesis and offspring health are related to maternal diet composition, which is an important determinant of the balance between antioxidants and ROS (Moore and Davies 2005). Maternal malnutrition, particularly in cases of deficiency of antioxidants, vitamins and trace elements, negatively impacts organogenesis and plays a major role in programming the offspring

susceptibility to oxidative stress and diseases (Franco et al. 2007b). Copper-deficient embryos have low SOD activity and high concentrations of $^{\bullet}O_2^-$ (Hawk et al. 2003). Copper deficiency before and during early mouse pregnancy has been shown to increase the number of embryonic resorptions and the incidences of embryonic brain and heart developmental anomalies (Beckers-Trapp et al. 2006). Zinc deficiency or even suboptimal zinc nutrition during pregnancy can lead to long-term adverse effect on rat brain function through deregulation of the transcription factors activator protein 1 (AP-1) and nuclear factor κB (NF-κB) (Aimo et al. 2010).

7.3.2 Unhealthy Lifestyle Behaviours

Unhealthy dietary habits, including consumption of low-cost fast/processed foods, overconsumption of refined carbohydrates and high consumption of red meat, before and during pregnancy are important determinants of adverse prenatal developmental outcomes and risk in developing NCDs in adulthood (Zhang et al. 2006; Uusitalo et al. 2009; Bao et al. 2013; Todd et al. 2015; Marí-Sanchis et al. 2017). Adult offspring of mice who consume a diet rich in saturated fats during pregnancy are at increased risk of oxidative-mediated chronic health disorders, such as hyperglycaemia, insulin resistance, obesity and hypertension (Liang et al. 2009). Placentas from high-saturated-fat diet dams demonstrated oxidative stress-induced labyrinthine endothelial cellular damage (Liang et al. 2010). A diet high in bread, confectioneries and soft drinks and low in fish and vegetables during pregnancy has been shown to be associated with IUGR and small birth weight (Okubo et al. 2012). The major unhealthy lifestyle behaviours that adversely affect the health of pregnant women and their foetuses are tobacco smoking, alcohol consumption and drug abuse. Exposure to alcohol, nicotine and medical drugs during pregnancy is associated with a significant health risk to the developing foetus because these chemicals cross the placental barrier and adversely affect early life development at least in part through oxidative stress (Kuhnert and Kuhnert 1985; Luck et al. 1985; Dodic et al. 1998; Morrison et al. 2004, 2005; Rossner et al. 2009). Alcohol consumption (Miller 2003; Simpson et al. 2005; Snow and Keiver 2007), cigarette smoking (Leonardi-Bee et al. 2008) and medication (Thompson et al. 2009b) impact foetal development and lead to IUGR (Kalinka and Hanke 1996; Horta et al. 1997; Bada et al. 2005) and adversely affect offspring health outcomes (Feng et al. 2005).

Perinatal exposure (from gestation day 40 to 1 year of age in childhood) of the non-human primate model *Macaca mulatta* to tobacco smoke (1 mg/m^3 total suspended particulates) increases vascular oxidative stress and mitochondrial damage and dysfunction as evidenced by a decrease of mitochondrial antioxidant capacity and mitochondrial copy number in aortic tissues (Westbrook et al. 2010). Exposure of mice to cigarette smoke for 6 weeks before mating, throughout gestation and lactation, leads to oxidative stress, decreased mitochondrial antioxidant capacity, mitochondrial dysfunction, kidney underdevelopment and functional abnormalities in adulthood (Al-Odat et al. 2014; Stangenberg et al. 2015).

7.3.3 Exposure to Environmental Pollutants

Human exposure to many environmental pollutants becomes inevitable due to their ubiquitous occurrence in air, water, soil, vegetables, food, industrial and domestic products, plastic products, cosmetics and medication. Exposure to pollution, almost all in developed and developing countries, is associated with increased morbidity and mortality (Dominici et al. 2007; Chen and Kan 2008; Wong et al. 2010; Qian et al. 2010; Romieu et al. 2012) from respiratory (Del Donno et al. 2002), cardiovascular (Bai et al. 2007) and central nervous system (Genc et al. 2012) diseases. Developmental toxicity during pregnancy caused by various environmental pollutants has become also a major health concern. Exposure of the developing embryo and foetus to multiple environmental pollutants during pregnancy could adversely affect the prenatal developmental trajectory in a cumulative dose-additive manner (Wigle et al. 2008; Rider et al. 2010). The main recognized disorders and complications linked to such exposure are embryonic mortality, foetal loss, IUGR preterm birth, birth defects, childhood diseases, neuropsychological deficits, premature or delayed sexual maturation and certain adult cancers (Wigle et al. 2008). Maternal exposure to environmental pollutants before and during pregnancy occurs through the consumption of contaminated food, water and beverages that may affect developmental and health outcomes (Al-Gubory 2014b).

The mother is exposed to many environmental pollutants during pregnancy, including heavy metals and pesticides (Roels et al. 1978; Chand et al. 2014; Ding et al. 2015; Naksen et al. 2015; Tyagi et al. 2015), endocrine disruptors (Amaral Mendes 2002; Sweeney 2002; Miller et al. 2004), ambient air particulate matter, certain disinfectants and occupational pollutants (Rocha et al. 2008; Veras et al. 2008; Vieira 2015). The foetus is a target for transplacental environmental pollutants especially during the period of organogenesis (Rossner et al. 2009; Yurdakök 2012; Dewan et al. 2013; Vizcaino et al. 2014; Zhang et al. 2017) and is fairly susceptible to oxidative damage due to its inadequate antioxidant defence systems (Davis and Auten 2010).

Adult offspring of rats exposed to 30 ppm of cadmium during pregnancy exhibit abnormal heart morphology characterized by a concentric left ventricular hypertrophy (Ronco et al. 2011). Exposure of rats to a mixture of arsenic, cadmium and lead in concentrations detected in groundwater of India (0.38, 0.098, and 0.22 ppm) from gestation day 5 to postnatal day 180 results in post-weaning and early adulthood augmentation of amyloid beta (Aβ) in the frontal cortex and hippocampus (Ashok et al. 2015). Persistent organic pollutants (POPs) are classes of compounds including organochlorine pesticides. They are persistent in the environment, resist chemical and biological degradation and can be transported in air and water and released far from their place of production, where they accumulate in terrestrial and aquatic ecosystems. POPs possess toxic and endocrine-disrupting potential (Bonefeld-Jørgensen et al. 2014) and affect animal development and growth (Anselmo et al. 2011; Lyche et al. 2011; Gill et al. 2013).

The global use of human-made low-cost chemicals that disrupt the endocrine system (endocrine disruptors) is on the rise, causing serious environmental and health problems. Developmental toxicity during pregnancy caused by various endocrine disruptors has become also a major health concern because these pollutants

have the ability to mimic endogenous steroid hormones and interfere with endocrine processes during pregnancy (Singleton and Khan 2003). Endocrine disruptors, including triclosan (TCS), bisphenol A (BPA) and certain pesticides, are present in many common consumer products such as cosmetics, pharmaceuticals, plastic, household products or food packaging (Orth 1980; Simon 1986; Adolfsson-Erici et al. 2002; Singer et al. 2002; Schettler 2006; Klingmüller and Alléra 2011). Exposure of pregnant mice to BPA is associated with underdevelopment of the foetal brain, kidney and testis (Kabuto et al. 2004). Treatment of mice for 5 days with doses of BPA below the no-observed-adverse-effect concentration (0.05 and 1.2 mg/kg/day) decreases the expression of glutathione peroxidase 3 (GPX3) and increases ROS and MDA in the liver that ultimately leads to mitochondrial dysfunction and liver damage (Ooe et al. 2005). Exposure of rats to TCS before mating and during pregnancy and lactation impairs maternal thyroid homeostasis and offspring pubertal development (Rodríguez and Sanchez 2010). Exposure of pregnant rats to TCS induces maternal, foetal and early neonatal hypothyroxinaemia (Paul et al. 2012).

Chloroacetonitrile, a disinfection by-product of chlorination of drinking water, when administered to pregnant mice between days 6 and 18 of pregnancy (25 mg/kg), crosses the placental and foetal blood-brain barrier, enhances oxidative stress and apoptosis in foetal brain and induces a 22% reduction in brain weight of foetuses (Ahmed et al. 2005). Exposure to chloroacetonitrile between days 6 and 18 of pregnancy (25 mg/kg) induces redox imbalance (decrease in the ratio of the reduced to oxidized form of glutathione, GSH/GSSG), oxidative stress (increased MDA and 8-hydroxy-2′-deoxyguanosine, 8-OHdG), apoptosis and histopathological changes (vacuolated cytoplasm, karyolysis and karyorrhexis, depletion of glycogen content) in foetal livers of mice (Abdel-Naim et al. 2009).

Ambient particulate matter (PM) promotes DNA damage through the redox cycling-based generation of ROS (Kumagai et al. 1997) leading to oxidative stress and tissue damage (Li et al. 2002). Short-term inhalation exposure to high concentrations of ambient air fine particles (0.1–2.5 μm) increases ROS concentrations and promotes oxidative stress and mild damage to the lungs and heart of rats (Gurgueira et al. 2002). Rats breathing concentrated ambient particles exhibit lung oxidative stress, as evidenced by the accumulation of TBARS (90 ± 15 pmol/mg protein; sham control 50 ± 5 pmol/mg protein) and oxidized proteins (1.6 ± 0.4 nmol/mg protein; sham 0.70 ± 0.02 nmol/mg protein) in their lungs (Rhoden et al. 2004). A cohort study including 74,671 women living in four Chinese regions reveals that exposure to air pollution increases the risk of low birth weight (Wang et al. 1997).

An International Collaboration on Air Pollution and Pregnancy Outcomes (ICAPPO) with more than three million singleton term births proved the link between maternal exposure to particulate air pollution (PM10 and PM2.5) and low birth weight (<2500 g at 37–42 weeks of gestation), across 14 centres from nine countries from North America, South America, Europe, Asia and Oceania (Dadvand et al. 2013). Mice exposed to diesel exhaust (DE) particulates for 3 weeks (≈300 μg/m^3 PM2.5 for 6 h/day, 5 days/week) from gestation day 0 to postnatal day 21 increased offspring susceptibility to cardiac hypertrophy, systolic failure, myocardial fibrosis and pulmonary congestion (Weldy et al. 2013). It is important to note that neonatal exposure to DE particulates does not predispose

mice to transverse aortic constriction-induced cardiac hypertrophy and heart failure in adulthood (Liu et al. 2016).

7.4 Antioxidants in Prevention of Prenatal Developmental Disorders

We are learning from experiences in rats about the beneficial effects of vitamin C, vitamin E, vitamin A, selenium or polyphenols in embryonic, foetal and placental development, as well as the prevention of oxidative stress and intrauterine and postnatal complications (Table 7.2). Nevertheless, the effectiveness of selected antioxidants to prevent prenatal development disorders in human is inconclusive (Tarin et al. 2002; Rumbold and Crowther 2005a, b). Vitamin C (1000 mg) and/or vitamin E (400 IU) supplementation to women ($n = 100$) with clinical risk factors (previous preeclampsia, chronic hypertension, pregestational diabetes, or multifoetal gestation) does not prevent preeclampsia (Beazley et al. 2005). Supplementation with Vitamin C (1000 mg) and vitamin E (400 IU) during pregnancy does not reduce the risk of preeclampsia in nulliparous women ($n = 935$) and the risk of IUGR or other serious health outcomes in their infants (Rumbold et al. 2006). In a randomized, placebo-controlled, double-blind clinical trial conducted at four Brazilian sites, treatment with both vitamin C (1000 mg) and vitamin E (400 IU) failed to reduce the rate of preeclampsia among patients ($n = 739$) with chronic hypertension and/or prior preeclampsia (Spinnato et al. 2007). Intake of retinol (662 μg), β-carotene (3407 μg), vitamin C (172 mg), vitamin E (13.4 mg), selenium (77.4 μg), zinc (16.2 mg) or manganese (6.44 mg) by pregnant women ($n = 3730$), who delivered an infant with genetic susceptibility to type 1 diabetes, does not protect their children from development of advanced β-cell autoimmunity (Uusitalo et al. 2008). This large-scale study was carried out in Finland, between October 1997 and December 2002 as part of the population-based birth cohort of the "Type 1 Diabetes Prediction and Prevention Project" in which maternal antioxidant intake during pregnancy was assessed postnatally with a self-administered food frequency questionnaire, which contained a question about consumption of dietary supplements.

The failure to prevent prenatal developmental disorders with vitamins and/or trace elements supplementation may be due to the dosage of antioxidants, the duration of treatment or the administration of imbalanced dietary antioxidants. Mean birth weight is relatively higher (3092 ± 190 g, $n = 1328$ recruited women) with multiple micronutrient supplementation (vitamin A, 800 μg; vitamin D, 200 IU; vitamin E, 10 mg; vitamin C, 70 mg; vitamin B1, 1.4 mg; vitamin B2, 1.4 mg; vitamin B3, 18 mg; vitamin B6, 1.9 mg; vitamin B12, 2.6 mg; folic acid, 400 μg; iron, 30 mg; zinc, 15 mg; copper, 2 μg; selenium, 65 mg; and iodine, 150 μg) than with only iron (60 mg) and folic acid (400 μg) supplementation (3025 ± 205 g, $n = 1222$ recruited women), which corresponds to a 14% decrease in the incidence of low birth weight (Zagré et al. 2007). Original data from 12 randomized, controlled trials in low-income countries (Bangladesh, Burkina Faso, China, Guinea-Bissau, Indonesia, Mexico, Nepal, Niger, Pakistan and Zimbabwe) indicate that maternal

Table 7.2 Studies examining the beneficial effects of antioxidants in embryonic, foetal and placental development and prevention of intrauterine and postnatal complications

Antioxidant	Study model	Beneficial effects	Reference
Vitamin C	Pregnant diabetic rats	Reduction of congenital malformations	Simán and Eriksson (1997)
Vitamin E	Pregnant diabetic rats	Decrease in plasma MDA concentrations Increase in GSH contents and SOD activity in the liver and uterus	Kinalski et al. (1999)
Vitamin E and Vitamin C	Pregnant diabetic rats	Improvement of foetal morphology	Cederberg and Eriksson (2005)
Folic acid and vitamin E	Pregnant diabetic rats	Prevention of diabetes-induced embryonic malformations and résorptions	Gäreskog et al. (2006)
Vitamin A, vitamin E, vitamin C and selenium	Western diet-fed pregnant rats	Restoration of the antioxidant balance Decrease of adiposity in offspring	Sen and Simmons (2010)
Selenium	Selenium-deficient pregnant rats	Prevention of selenium-deficient-induced mortality at birth	Nogales et al. (2013)
Polyphenol-rich plant extracts	Pregnant diabetic rats	Decreased hyperglycaemia	Yessoufou et al. (2013)
Resveratrol	Pregnant nonhuman primates fed a Western-style diet	Improvement of glucose tolerance Increase uterine artery volume blood flow Decrease placental inflammation and liver triglyceride deposition	Roberts et al. (2014)
Resveratrol	Rat offspring exposed to prenatal hypoxia	Improvement of cardiac recovery from ischemia-reperfusion and oxidative stress injuries	Shah et al. (2015)
Resveratrol	Low protein-fed pregnant rats	Prevention of low protein-induced oxidative stress in the placental, foetal and maternal liver	Vega et al. (2016)

supplementation with multiple micronutrients during pregnancy slightly reduces the prevalence of low birth weight (Fall et al. 2009).

7.5 Plant-Based Diets in Prevention of Prenatal Developmental Disorders

Plant diets, including foods, beverages, spices and herbs with varied and balanced antioxidants (Carlsen et al. 2010), are more effective than the consumption of some antioxidants or even supplementation with high doses of a single antioxidant. Therefore, identification of plant-based foods favouring the prenatal development

should be a priority in prenatal health research and disease prevention. Evidence from experimental and epidemiological studies indicates that dietary phytochemicals and plant-based foods have a very wide-ranging beneficial influence on the body to ensure good health. Many studies reported hereafter might provide a rationale for future investigations into the beneficial effects of medicinal plants, plant-based functional foods, beverages, diet quality and healthy dietary habits on prenatal development and health outcomes.

Toki-shakuyaku-san, a Japanese herbal medicine known to remove free radicals (Stefek and Benes 1994), has proved to be effective in reducing the adverse effects of excess $^{\bullet}O_2^-$ on the endometrium during implantation in mice (Ota et al. 1999). The presence of flavonoids in urine and carotenoids in plasma of women during early pregnancy has been shown to reflect the intake of fruits, vegetables, juice and tea (Brantsaeter et al. 2007). The presence of polyphenolic compounds in foetal organs after maternal ingestion of these antioxidants offers the possibility of antioxidant periconceptional supplementation to support conceptus development and survival (Chu et al. 2007). Aqueous extract of the date palm fruits (*Phoenix dactylifera* L.) has been shown to contain antioxidant and antimutagenic properties (Vayalil 2002). Fruit of the date palm contains a wide array of phenolic compounds and carotenoids (Al-Farsi et al. 2005; Hong et al. 2006) with potential human health benefits (Vayalil 2012). Consumption of date fruit late in pregnancy has been shown to positively affect the outcome of labour and delivery (Al-Kuran et al. 2011).

The extra virgin olive oil is a natural source of flavonoids, carotenes and tocopherols that are believed to generate beneficial effects in human health (Servili et al. 2009). Early pregnancy olive oil supplementation has been shown to reduce embryonic mortality and/or malformation rate in diabetic rats at least in part through pathways that regulate prostaglandins and NO• production by embryonic and decidual tissues (Higa et al. 2010). Foetal lungs of female foetuses of diabetic rats fed during pregnancy diets supplemented with 6% olive oil or 6% safflower oil show decreased cholesterol and cholesteryl ester concentrations and increased expression of the reverse cholesterol transporter ATP-binding cassette A1 (Kurtz et al. 2014). A diet enriched in olive oil increases the expression of peroxisome proliferator-activated receptors (PPARs) that regulate antioxidant and anti-inflammatory pathways in embryos and decidua from diabetic rats (Higa et al. 2014). Hearts of adult offspring from diabetic rats exhibit increases in triglycerides in males and phospholipids, cholesterol and free fatty acids in females; such alterations can be inhibited with olive oil-enriched diet (6%) given during pregnancy (Capobianco et al. 2015).

Honey is a natural plant product with potential antioxidant capacity (Gheldof and Engeseth 2002). Propolis is a honeybee product known to be beneficial for human health (Khalil 2006). Honeys and propolis, irrespective of floral sources, contain a wide range of antioxidant compounds, mainly phenolic acids and flavonoids (Gheldof et al. 2002; Orsolić et al. 2006). In vitro study has shown that honey has anti-inflammatory property (van den Berg et al. 2008) and inhibits lipid peroxidation at least in part by ROS scavenging and metal chelating (Hegazi and Abd El-Hady 2009). Propolis extract has been shown to increase SOD activity and inhibit lipid peroxidation in rats with diabetes mellitus (Fuliang et al. 2005). Supplementation of honey (1.2 g/kg body weight) daily from day 1 of pregnancy

until delivery reduces the adverse effects of restraint stress (three times per day starting on day 11 of pregnancy) on the weight of reproductive organs and sperm parameters in male rat offspring (Haron and Mohamed 2016).

Tea, the world's most important beverage after water, is a pleasant drink, cheap, easy to prepare and therefore accessible to anyone in the world. In humans, an increase in plasma antioxidant capacity has been demonstrated following the consumption of black tea (Langley-Evans 2000). Black and green teas are rich sources of dietary polyphenolic compounds (Rechner et al. 2002; Stewart et al. 2005). Polyphenols from either green or black tea have been shown to reduce ROS production from endothelial cells, at least in part, by downregulation of NADPH oxidase and upregulation of CAT (Ying et al. 2004). Experimental and epidemiological evidence suggests that black or green tea has beneficial health effects in lowering the risk of heart diseases (Gardner et al. 2007; Basu and Lucas 2007). The polyphenols from black tea and green tea exhibit protective action against the arsenite-induced depletion of antioxidant enzymes in mice liver tissue (Sinha et al. 2010). Green tea polyphenols at doses of 15 μM increase mRNA expression encoding SOD1, GPX and CAT genes and decrease apoptosis in cultured cow blastocysts (Wang et al. 2013). Moreover, cows receiving embryos treated in vitro with 15 μM green tea polyphenols have higher pregnancy rates on day 30 (34.8% vs. 28.6%) and day 60 (34.8% vs. 23.9%) than those receiving control embryos (Wang et al. 2013). It is important to note that in Chinese pregnant women (n = 8775), tea drinking during early pregnancy is not associated with an increased risk of preterm birth or abnormal foetal growth (Lu et al. 2017).

Pomegranate fruit products, mainly peel and juice, received great attention over the last decade because of their potential disease prevention (Lansky and Newman 2007) and high content in polyphenolic phytochemicals (Henning et al. 2014). Data from animal studies showed that pomegranate peel extract (50 mg/kg in terms of catechin equivalents) counteracts the inhibitory effect of carbon tetrachloride (2.0 g/kg of body weight) on activities of SOD, GPX and CAT (Chidambara Murthy et al. 2002) and protects the rat liver from fibrosis and lipid peroxidation induced by experimental biliary obstruction (Toklu et al. 2007). High doses of pomegranate peel extract (200 and 400 mg/kg body weight/day) protect the rat liver against pentachlorophenol-induced oxidative stress and cytogenetic toxicity (Agha Fatma et al. 2013). Treatment of mice with pomegranate peel extract (6 mg/day per mouse) for 4 weeks reduces serum level of cholesterol (total and low-density lipoprotein, LDL) induced by a high-fat (HF) diet and counteracts the HF-induced expression of inflammatory markers both in the colon and the visceral adipose tissue (Neyrinck et al. 2013). Oral gavage with 100 μL of pomegranate peel extract (300 mg/kg body weight) daily over 5 days attenuates inflammation of the mice jejunum induced by *E. papillata* infections (Amer et al. 2015). Pomegranate juice reduces oxidative stress in human placental villi in vivo and limits stimulus-induced apoptosis of trophoblast in culture (Chen et al. 2012).

Supplementation with pomegranate peel extract (50 mg/kg body weight/day) for 30 days protects rats from mercuric chloride (5 mg/kg body weight in 0.9% NaCl)-induced oxidative damage (Kumar et al. 2013). Intake of pomegranate juice by pregnant women with singleton pregnancies from 35 to 38 weeks of gestation until

delivery decreases oxidative stress in term placentas (Chen et al. 2012). In this study, pomegranate juice reduces oxidative stress and apoptosis in term villous explants and primary trophoblast cultures exposed to hypoxia, while punicalagin, but not ellagic acid, both prominent polyphenols in pomegranate juice, reduces oxidative stress and apoptosis in cultured syncytiotrophoblasts. Treatment of day 8.5 mouse embryos with 20 μmol of pomegranate punicalagin inhibits glucose-induced neural tube defect formation, suggesting that punicalagin supplements could mitigate the teratogenic effects of hyperglycaemia in the developing embryo and prevent diabetes-induced neural tube defects (Zhong et al. 2015).

Curcumin, a yellow pigment and polyphenolic compound of turmeric (*Curcuma longa*), is a commonly used spice with potential anti-inflammatory effects through the downregulation of inflammatory transcription factors, such as nuclear factor kappaB (NF-κB); enzymes, such as cyclooxygenase 2 (COX2) and 5-lipoxygenase (5-LOX); and cytokines, such as Tumor necrosis factor (TNFα) IL1 and IL6 (Aggarwal and Sung 2009). Curcumin may have a therapeutic role with regard to neurodegenerative diseases, cancer, type 2 diabetes, coronary heart diseases and inflammation (Margină et al. 2015). Treatment of male rats with 20 mg/kg body weight of cisplatin, an important anticancer agent useful in treatment of various cancers, increases hepatic MDA levels and reduces hepatic SOD and CAT activities and NADPH oxidase expression (Palipoch et al. 2014). Pretreatment of rats with a single dose of α-tocopherol (250 mg/kg body weight) and curcumin (200 mg/kg body weight) increases SOD and CAT activities and reduces MDA and gene expression of NADPH in the liver (Palipoch et al. 2014).

Elevated levels of homocysteine (Hcy), a common blood thiol-containing amino acid, may lead to increased generation of vascular $^{\bullet}O_2^-$ levels, which results in the inactivation of the vasorelaxant effect of $NO^{\bullet}$ and leads to endothelial dysfunction (Weiss et al. 2003). Treatment of porcine coronary arteries with curcumin (5 μmol/L) reverses the endothelial dysfunction (impairment of endothelium-dependent vasorelaxation) induced by Hcy (50 μmol/L), at least partly by inhibition of Hcy-induced $^{\bullet}O_2^-$ production and eNOS downregulation (Ramaswami et al. 2004). Treatment of male and female rats with methionine (1 g/kg body weight) for 30 days increases total cholesterol, triglycerides, LDL cholesterol (LDL-C) and serum Hcy levels and cardiac TBARS content (Kapoor et al. 2008). Curcumin (200 mg/kg body weight) treatment in methionine-treated rats decreases the total cholesterol, triglycerides, LDL-C and serum Hcy levels and cardiac TBARS content (Kapoor et al. 2008). Curcumin (20 μmol/L) reduces high glucose-induced neural tube defect formation in embryonic day 8.5 mouse embryos by blocking embryonic oxidative stress and caspase activation (Wu et al. 2015). Treatment of pregnant mice with curcumin (0.36 mg/kg) improves the lipopolysaccharide (LPS)-induced deficient trophoblast invasion and spiral artery remodelling (Gong et al. 2016). In mice model of placental inflammation and prenatal developmental disorders induced by LPS, curcumin given during pregnancy inhibits the expression of placental pro-inflammatory cytokine expression, such as TNF-α, IL-1β and IL-6, improves foetal and placental development and decreases foetal resorption rate (Zhou et al. 2017).

An example worth highlighting is the health benefit effects of functional foods in the Mediterranean diet (MD) (Ortega 2006), which is well known as a rich source

of many antioxidants, mainly vitamins, trace elements, carotenoids and polyphenols (Dai et al. 2008). This diet is composed of high intake of fruit, vegetables, cereals, nuts and extra virgin olive oil; high to moderate consumption of fish; moderate consumption of dairy products, and wine; and low consumption of meat and meat products. Adherence by the couple to the MD has been shown to increase the probability of pregnancy, improve fertility (Vujkovic et al. 2010) and decreases the risk of premature labour and gestational diabetes (Barger 2010; Toledo et al. 2011). Hcy is produced from the dietary amino acid methionine. Deficiencies of B vitamins required for the transsulfuration or remethylation pathways of Hcy result in an elevation of blood Hcy levels (hyperhomocysteinaemia), which is an important risk factor for human chronic diseases (Perla-Kajan et al. 2007).

Hyperhomocysteinaemia and oxidative stress are etiological factors for pregnancy disorders, such as abortion and preterm birth in women (Micle et al. 2012). Plant components in the MD have significant antioxidant activity and anti-inflammatory actions, serving as the basis for therapeutic or preventative nutritional strategies to counteract hyperhomocysteinaemia-induced oxidative stress and its associated prenatal disorders and complications. Neonates whose mothers consumed low MD present low lipoprotein and Hcy levels at birth (Gesteiro et al. 2015). MD is inversely associated with risk of developing hypertensive disorders of pregnancy (Schoenaker et al. 2015), whereas low adherence to the MD is associated with higher risk of gestational diabetes mellitus and hypertensive disorders of pregnancy (Schoenaker et al. 2016). MD could therefore be a valuable strategy to adopt so as to prevent prenatal development disorders and complications related to ROS-induced oxidative stress.

7.6 Conclusions

The establishment and successful outcome of pregnancy in the birth of a healthy offspring are the sine qua non preconditions for effective long-term adult health. Maternal exposure to adverse environmental conditions before and during pregnancy impacting prenatal development has long-term effects on offspring health and increases the risk of men and women NCDs. Therefore, an understanding of the mechanisms leading to adverse prenatal development outcomes along with constraining environmental factors during the periconceptional period will enable the development of valuable therapeutic strategies.

Antioxidant-rich diets may reduce gestational abnormalities in women with a high risk of adverse prenatal developmental and pregnancy outcomes. Animals are essential for understanding the in utero origin and evolution of the pathology in humans. They are also required to verify the effectiveness and safety of nutritional interventions before the implementation of clinical trials aiming at improvement of women health and prevention of early life programming diseases. Experimental animal models remain valuable for evaluating new nutritional therapies and should prove the safety and effectiveness of plant antioxidants as a reliable treatment of environmental factor-induced oxidative stress, female infertility and prenatal

developmental disorders and complications, with the expectation that the protective effects of dietary antioxidants gained might be extrapolated to humans.

In view of the beneficial health impact of fruit and vegetables, which are rich in multiple and balanced antioxidants and essential trace elements, I can expect that plant-based functional foods and beverages, diet quality, healthy dietary habits and education will provide a preventative therapeutic strategy against oxidative stress and associated prenatal developmental disorders and complications. Human observational studies in the natural and/or altered environmental conditions and controlled interventions in humans will be needed to understand the beneficial effects of fruit and vegetable antioxidants in prenatal development and prevention of early life programming diseases.

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Chapter 8
Antioxidants Against Environmental Factor-Induced Oxidative Stress

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Abstract Reactive oxygen species (ROS) are ubiquitously produced within the cell as natural by-products of aerobic respiration and energy production. Human-made pollutants present in our environments also add additional burden to biological systems and could lead to enhanced ROS production. Overproduction of ROS and/or inadequate antioxidant defense mechanisms can induce oxidative damage to a wide range of biomolecules including lipids, proteins, and nucleic acids that adversely affect organ function and health outcome. Exposure to environmental factors such as ultraviolet light, ionizing radiation, heavy metals, pesticides, and air pollutants is thought to adversely impact the function of biological systems by virtue of ROS generation. These factors are important contributor of a wide range of diseases, such as respiratory, neurological, heart, and reproductive diseases, as well as cancer. Although there are several antioxidant enzymes within cells of biological tissues that scavenge ROS, some important antioxidants cannot be synthesized by cells and must be taken in the diet to counteract oxidative damage. In this chapter, we discuss the protective roles of dietary antioxidants in the prevention of oxidative injuries induced by environmental pollutants, the scope and limitation of antioxidant therapies used against environmental factor-induced oxidative stress, and the discrepancies between the results of experimental models and epidemiologic studies. The overarching focus of this chapter is to stress the importance of nutrition and dietary antioxidants as potential modulators of disease risks associated with exposure to multiple environmental pollutants.

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8.1 Introduction

One of the most important Earth events was the change in atmospheric gas composition due to increasing concentration of free oxygen (O_2) as a result of photosynthesis by ancestors of cyanobacteria approximately 2500 million years ago (Lyons et al. 2014). This important past event is what we commonly refer to as an "environmental change." One can ask what is an environment? A classic definition is "*The combination of all the conditions external to the genome that potentially affect its expression and its structure*" (Griffiths 2012). The ability of aerobic organisms to adapt themselves to new environmental conditions occurs as a result of interactions between genes and the environment. Therefore, the metabolic systems of the majority of living organisms, both prokaryotic and eukaryotic, have adapted to generate energy, thanks to O_2. Cellular energy is derived from the transport of electrons from oxidizable organic molecules, thereby reducing O_2 in the mitochondrial respiratory chain. This process constitutes one of the most important intracellular sources of reactive oxygen species (ROS), including free radicals in eukaryotic cells (Dröse and Brandt 2012), but paradoxically induces oxidative cellular damage.

Over the past decades and currently, human activities contribute to climate change by causing changes in Earth's atmosphere of greenhouse gases, mainly carbon dioxide, nitrous oxide, and methane, which adversely impact our environment and health. In addition, exposure to human-made pollutants, mainly particulate matters, polycyclic aromatic hydrocarbons, heavy metals, pesticides, as well as exposure to ultraviolet (UV) light and/or ionizing radiation, may lead to oxidative stress and chronic diseases. These environmental factors are important contributor of a wide range of pathologies, such as neurological diseases (Cannon and Greenamyre 2011), respiratory diseases (Anderson et al. 2012), cancer (Pogribny and Rusyn 2013), cardiovascular diseases (Kirkley and Sargis 2014), obesity (Ghosh et al. 2014), allergic diseases (Elshabrawy et al. 2014; Yi et al. 2015), and reproductive disorders and diseases (Al-Gubory 2014; Hauser et al. 2015). Oxidative damage can result either from the direct effects of pollutants or indirectly due to ROS formation and accumulation when toxic agents are metabolized prior to elimination. In the context of current human activities, and consequently a release to our environment of significant quantities of organic and nonorganic pollutants, prevention strategies to minimize the adverse effects of pollutants on the environment and human health are a challenge. In this chapter, we discuss the protective roles of dietary antioxidants against toxic effects of environmental pollutants, the scope and limitation of antioxidant therapies used against environmental factor-induced oxidative stress, and the discrepancies between the results of experimental models and epidemiologic studies.

8.2 Dietary Antioxidants

Dietary antioxidants play a fundamental role in maintaining redox balance and reducing the incidence of free radical-induced damage. The use of natural plant products to reduce oxidative stress is a strategy that is currently used to prevent or protect against the oxidative damage (Kapiszewska et al. 2005). Experimental dietary studies in humans have revealed the capacity of these plant foods and some of their constituents to modify antioxidant pathways, detoxification enzyme profiles, and the immune system as well as alter cholesterol and steroid hormone concentrations and metabolism (Lampe 1999). A number of studies have indicated that plant-derived components and extracts can be considered for use as prophylactic agents in cancer. Plant extracts with high antioxidant indices can mediate their anticarcinogenic effects, at least in part, by acting as free radical scavengers and metal chelators (Neergheen et al. 2010). Numerous animal and human studies have revealed that dietary components of plant origin have chemopreventive properties against chronic diseases (Boeing et al. 2012). These protective capabilities result from the wealth of nutrients, such as vitamins, minerals, and fiber. Within this large group are carotenoids, polyphenols, alkaloids, nitrogen compounds, and organosulfur (Liu 2004).

8.2.1 Vitamin C

Vitamin C is an essential cofactor for several enzymes and participates in the recycling of vitamin E (Beyer 1994; Grosso et al. 2013). Although the majority of animals are able to endogenously synthesize significant quantities of vitamin C, humans have lost this capability due to a series of mutations of the gene encoding L-gulono-gamma-lactone oxidase, which catalyzes the last step in the synthesis of ascorbate (Grosso et al. 2013; Nishikimi et al. 1994). In humans, adequate levels of body vitamin C can be obtained through the consumption of fresh fruits and vegetables or vitamin supplements. Numerous factors contribute to vitamin C status in the body, including the vitamin C transporters, sodium-ascorbate cotransporter 1 and 2 (SVCT1, SVCT2), which regulate its bioavailability and concentration in plasma and tissues (Michels et al. 2013). Vitamin C exists in easily interconvertible reduced (ascorbate) and oxidized (dehydroascorbic acid) forms. Chemical activity is modified by O_2 oxidation, alkali, and high temperature (Chambial et al. 2013). Vitamin C participates in normal physiological functions, including the synthesis and metabolism of amino acids and catecholamines, the metabolism of cholesterol and its conversion into bile acids, and the reduction of iron to its ferrous form (Chambial et al. 2013). As powerful antioxidant, vitamin C protects the body from various deleterious effects of free radicals, pollutants, and toxins (Chambial et al. 2013). Preventive and therapeutic uses of vitamin C consist of its use at much higher concentrations than the recommended dietary allowances

via oral or intravenous administration. In a study of patients with various cancers who were administered vitamin C at high doses (ranging from 7.5 to 50 g), significant reductions in pro-inflammatory cytokines, including interleukin 1α (IL-1α), interleukin-2 (IL-2), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), and chemokines, such as eotaxin and C-reactive protein, were observed (Mikirova et al. 2012).

8.2.2 Vitamin E

Vitamin E, which exists in eight lipophilic forms, tocopherol α, β, γ, and δ and tocotrienol α, β, γ, and δ (Jiang 2014), is a lipid-soluble antioxidant that is essential for human health. Inadequate levels of vitamin E precipitate in neurological diseases (Ulatowski and Manor 2013). Vitamin E plays a role in the prevention of degenerative diseases (Borel et al. 2013). Although vitamin E scavenges peroxyl radicals, its bioavailability in biomembranes is affected by numerous factors, such as transport mechanisms involving α-tocopherol transport protein (α-TTP), which alter antioxidant efficiency (Massaeli et al. 1999; Niki 1987). The effects of dietary and pharmacological antioxidant supplementation have been intensively examined using vitamin E. Tocopherols and tocotrienols are potent antioxidants with lipoperoxyl radical scavenging activities that are mediated via hydrogen donation from the phenolic group on the chromanol ring (Jiang 2014). Various forms of vitamin E are found in plants, and some seeds, such as nuts and almonds, which are rich sources of α- and γ-tocopherol, the most abundant forms of vitamin E in tissues. In vivo studies have evaluated the efficacy of various forms of vitamin E in the control of oxidative stress and inflammatory processes in rodent models of cancer and respiratory illness (Jiang 2014). Some forms of vitamin E have anti-inflammatory effects. For example, γ-tocotrienol (γ-TE) participates in the regulation of pro-inflammatory cytokines via the modulation of key transcription factors. γ-TE increased CCAAT-enhancer-binding protein β (C/EBPβ) formation while inhibiting inflammatory process-related interleukin-6 formation and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and signal transducer and activator of transcription 6 and 3 (STAT6/STAT3) signaling activation in a model of lipopolysaccharide-induced inflammation (Kannappan et al. 2010; Wang and Jiang 2013; Wang et al. 2012).

8.2.3 Carotenoids

Carotenoids, abundantly present in fruits and vegetables, are a wide group of natural fat-soluble pigments (Britton et al. 2004). β-carotene, lycopene, zeaxanthin, and lutein, the most dietary carotenoids that provide health benefits (Johnson 2002), display antioxidant properties in lipid phases by quenching singlet molecular

oxygen ($^1O_2$) and free radicals (Böhm et al. 2012). Therefore, carotenoids are powerful antioxidants that can help to prevent some diseases (Mayne 1996). Carotenoids play a protective role in a number of ROS-mediated disorders, such as cardiovascular diseases, several types of cancer and related complications, photosensitivity, and neurological disorders (Fiedor and Burda 2014). Although, epidemiologic studies have reported that the consumption of lycopene-rich foods decreases the risk of cardiovascular diseases and cancers (Gerster 1997; Rao and Agarwal 2000), it is argued that carotenoid mixtures (Stahl et al. 1998) or β-carotene with vitamins E and C (Böhm et al. 1998) offers more synergistic cell protection against ROS production than treatment with one carotenoid. It is important to note that due to their structural attributes, carotenoids are highly susceptible to oxidation and degradation after their extraction from biological tissues, which limits their protective benefits (Boon et al. 2010).

8.2.4 Polyphenols

Polyphenols comprise one of the most widely distributed antioxidants in the plant kingdom, with over 8000 known compounds (Pandey and Rivzi 2009). Flavonoids are the most common group in the family of polyphenolic compounds, with over 5000 different structures that are present in fruits and vegetables, as well as plant-derived foods and beverages, such as olive oil, tea, and red wine. Major groups include flavones, flavonols, flavanones, isoflavones, anthocyanidins, and flavanols (Dreosti 2000). Mechanisms that are proposed to be involved in the beneficial health effects exerted by flavonoids are antioxidant effects, metal chelation, enzyme inhibition, and gene regulation (Erlejman et al. 2004). The effects exerted by flavonoids have been generally observed to result from a combination of different mechanisms. Tea catechins have been found to demonstrate antioxidant activity that is mediated by the neutralization or sequestration of free radicals, but they also regulate cellular enzyme activities by modifying the structure of the cell membrane (Caturla et al. 2003). Flavonoids act as antioxidants at low concentrations, exerting their effects against oxidation or free radical damage. In addition, the flavonoid radical formed (aroxyl) should be stable and display effective antioxidant function (Williams et al. 2004). To understand the potential of flavonoids to act as antioxidants in vivo, it is necessary to consider factors such as bioavailability and gastrointestinal tract interactions, as well as the influences of conjugation and metabolism (Croft 1998).

8.3 Cruciferous Vegetables and Antioxidants

The cruciferous or brassica families are widely geographically distributed. They include more than 330 genera and 3700 species, all of which were derived from a common ancestor (*Brassica olacerea*). The *Brassica* genus includes broccoli,

cabbage, cauliflower, Brussels sprouts, watercress, arugula, and radishes. Cruciferous crops have existed since ancient times. The Romans used cabbage, cauliflower, and broccoli mainly for medicinal purposes and, less commonly, as food (Herr and Büchler 2010). Currently, these vegetables are consumed worldwide due to their high nutritional and health values (Kapusta-Duch et al. 2012). In ancient times, watercress (*Nasturtium officinale*) was used as a medicinal plant rather than as a food. The Greeks considered watercress to be an expectorant and stimulant, while the Romans used it as a remedy for hair loss and the prevention of dandruff on the scalp. Long valued both as a food and as a medicinal herb, watercress grows in dense patches along the banks of clear streams and ditches, by springs, and in marshy meadows. The herb's greatest power is found in its sweetly sharp, pungent leaves, best gathered before flowering.

The beneficial effects of Brassica vegetables and diets on human health have been linked to the presence of antioxidants, mainly vitamins C and E, carotenoids, and polyphenols (Kapusta-Duch et al. 2012). In addition, the *Brassica* is the main dietary source of glucosinolates (GSLs). These compounds, also referred to as thioglycosides, are water-soluble organic anions responsible for the flavor and aroma of cruciferous vegetables. They are formed by a β-D-thioglycollate group, an oxime group-sulfated variable side chain that is derived from an amino acid (alanine, valine, leucine, phenylalanine, tyrosine, etc.). GSLs are synthesized and stored in plants as inactive precursors. Glucosinolate hydrolysis is conducted by an enzyme (β-thioglucosidase or myrosinase) that is physically separated from its substrate in intact plants. The enzyme is also present in the intestinal tracts of mammals, producing hydrolysis during digestion. Thus, different products are formed by means of enzyme action; among the most important are the isothiocyanates (ITCs), thiocyanates, nitriles, and indoles.

Several epidemiological studies have established an inverse association between a cruciferous vegetable-rich diet and the risk of developing lung or gastrointestinal tract cancers. In addition, evidence of possible breast, prostate, bladder, and pancreatic cancer protection exists (Higdon et al. 2007; Kim and Park 2009). The mechanisms underlying the beneficial effects of cruciferous vegetables may be linked to ITCs and indoles. These molecules participate in key pathways involved in the regulation of carcinogenesis and the development of chronic pathologies, such as antioxidant and detoxifying gene response induction, inhibition of CYP450 enzymes, inhibition of histone deacetylase, inhibition of tumor growth and angiogenesis, and induction of apoptosis and cell cycle arrest in neoplastic cells (Traka and Mithen 2009). Anti-inflammatory and antibacterial properties, as well as cardiovascular, renal and neuronal protection, have also been attributed to ITCs and indoles (Dinkova-Kostova and Kostov 2012). However, it is important to note that all of the reported effects are based on the dosage of the phytochemical (Hayes et al. 2008). These compounds behave as protective agents at low concentrations, generating adaptive responses, while at higher concentrations, they may induce toxicity, oxidative stress, and cell death.

Watercress contains an array of nutritional compounds, such as vitamin C, β-carotene, α-tocopherol, and GSLs (Palaniswamy et al. 2003; Martínez-Sánchez

et al. 2008). The main glucosinolate present in watercress is gluconasturtiin (GNT), which is derived from the amino acid phenylalanine. GNT is hydrolyzed via the enzymatic action of myrosinase to phenylethyl isothiocyanate (PEITC). Many cell culture and animal model studies, as well as human trials, have indicated that isothiocyanates may decrease the risk of developing cancer via several mechanisms, including phase I enzyme inhibition, increased phase II enzyme activity, cell cycle arrest, and apoptosis induction. PEITC is believed to inhibit phase I enzymes and induce phase II enzymes, thereby diminishing the incidence of nitrosamine-induced lung and esophageal cancers in rats (Rose et al. 2000). In addition, this isothiocyanate induces lipid peroxidation (Kassie and Knasmüller 2000). Boyd et al. (2006) studied the chemopreventive potential of a watercress extract in a human cell line derived from HT29 colon cancer in relation to the stages of the carcinogenic process. The results revealed that the extract inhibited hydrogen peroxide- and fecal water-induced DNA damage. In addition, the extract restricted the S phase of the cell cycle and inhibited the invasion of the matrix HT115 cells. Watercress extract modifies the lipid profile in hypercholesterolemic rats (Yazdanparast et al. 2008). A 1-month treatment with the plant extract decreased total cholesterol, triglyceride, and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) levels. Moreover, watercress supplementation was observed to increase the reduced glutathione (GSH) content and catalase (CAT) and superoxide dismutase (SOD) activities while decreasing lipid peroxidation. In humans, supplementation of watercress in the diet increases plasma lutein and beta-carotene and reduces lymphocyte DNA damage (Gill et al. 2007). Moreover, consumption of watercress has been reported to attenuate excessive exercise-induced DNA damage and lipid peroxidation (Fogarty et al. 2013). As far as we are aware, watercress is an aquatic plant that readily bioaccumulates heavy metals that may be found in contaminated aquatic systems (Beals and Byl 2014). Therefore, we must take certain precautions regarding the use of watercress as antioxidant-rich food.

8.4 Environmental Factor-Induced Oxidative Stress and Therapies

Under physiological conditions, antioxidant systems maintain control over cellular metabolism-induced ROS production. However, exposure to heavy metals and metalloids, ionizing radiation, pesticides, persistent organic pollutants, particulate matter, and some pharmacological drugs results in the generation of reactive metabolites, which sometimes exceed antioxidant defenses leading to oxidative stress. Epidemiological studies have linked environmental exposure-induced oxidative stress to several pathological conditions. Environmental factors, such as dietary deficiencies, lifestyle (smoking, alcohol use, drug abuse, etc.), and xenobiotic exposure, have been linked to diverse types of cancer (Reuter et al. 2010; Sosa et al. 2013), diabetes (Rytter et al. 2010; Nebbioso et al. 2012), skin diseases (Bickers and Athar 2006), cardiovascular diseases (Dusting and Triggle 2005; Pashkow 2011),

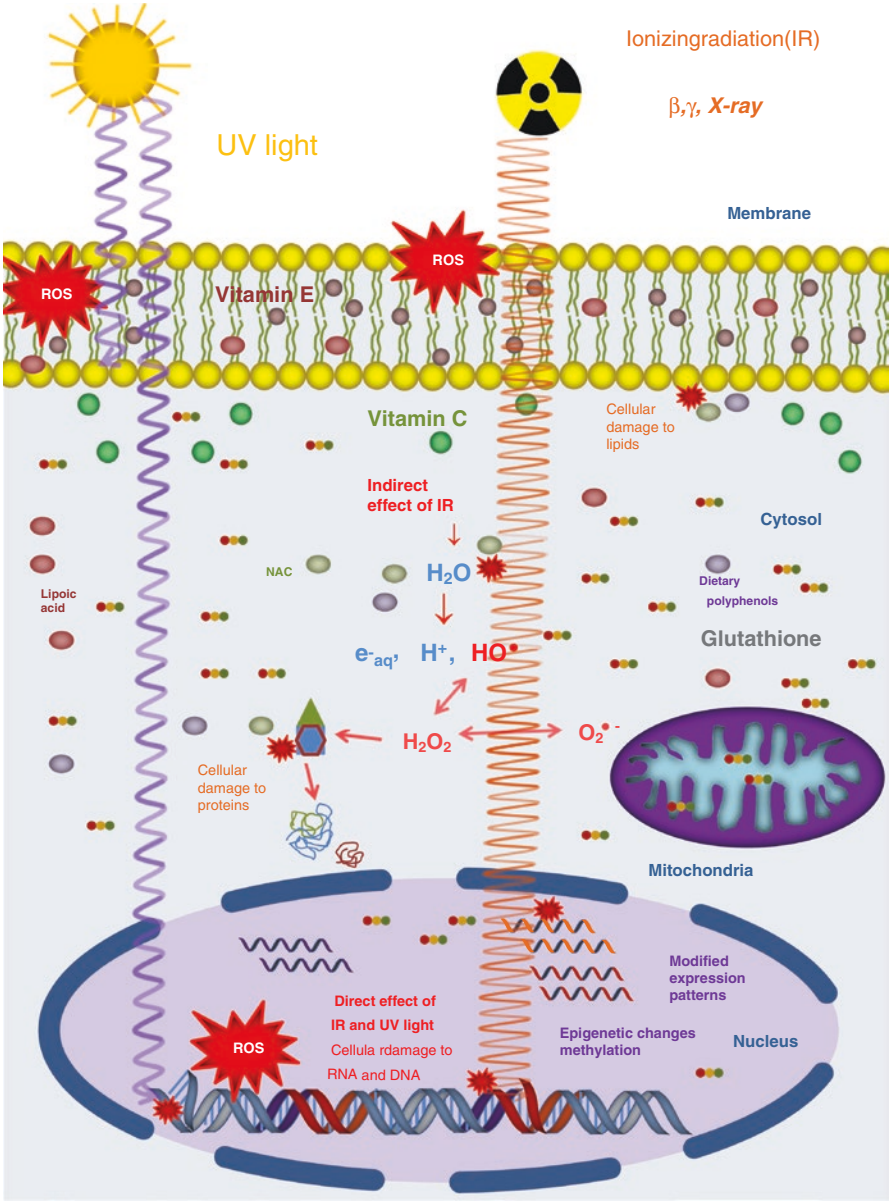
and neurodegenerative disorders (Yuan et al. 2007; Praticò 2008). Although several antioxidant defense systems may be depleted by excessive free radical production under chronic oxidative stress, some may be susceptible to manipulation, either pharmacologically or nutritionally. Thus, in the next section, we review some of the antioxidants that are widely used as nutritional or pharmacological therapeutic interventions following exposure to oxidant agents in the environment.

8.4.1 Ultraviolet Light and Ionizing Radiation

When skin is exposed to UV light, superoxide anions, hydroxyl radicals, singlet oxygen, and water and lipid peroxide formation have been linked to skin aging and tumor formation. Ionizing radiation (IR) increases the formation of one of the most reactive species, the hydroxyl radical ($^{\bullet}OH$), through the oxidation of water. The majority of these exposures occur in the low-dose range. However, exposure from medical and cosmetic procedures has increased considerably (Bernal et al. 2013). Recent evidence from the agouti mouse suggests that low-dose exposure to ionizing radiation during gestation increases DNA methylation in males, thereby inducing coat color changes. Maternal dietary supplementation with antioxidants, such as *tert*-butyl hydroquinone, seleno-L-methionine, vitamin C, vitamin E, α-lipoic acid, and *N*-acetylcysteine (NAC), mitigated these epigenetic changes (Bernal et al. 2013). Antioxidants and thiol compounds can be used as free radical scavengers before and during exposure to IR to prevent damage. The first antioxidant used in radioprotection was the amino acid cysteine. The cysteamine analog amifostine (WR 2721) is an organic thiophosphate used in human medicine for radioprotection. Other compounds, including nitroxides, such as tempol, have been developed to prevent oxidative stress and lipid peroxidation (Kuntić et al. 2013). Plant extracts with antioxidant properties, including carnosol, resveratrol, acerola, and catalpol

Fig. 8.1 Dietary supplementation with antioxidants and thiol compounds prevents oxidative damage induced by ultraviolet (UV) light and ionizing radiation (IR). UVA (320–400 nm) or UVB (290–320 nm) generates reactive oxygen species (ROS) such as superoxide anions ($^{\bullet}O_2^-$), hydroxyl radicals ($^{\bullet}OH$), singlet oxygen ($^1O_2$), and hydrogen peroxides (H_2O_2) that indirectly damage molecules through oxidation. Similarly, exposure to IR (β and γ particles and X-rays during medical procedures) increases the formation of ROS such as $^{\bullet}OH$ during water hydrolysis. Lipid peroxidation, protein oxidation, inactivation and degradation, and DNA oxidation occur by the excess of ROS. Changes in DNA methylation patterns and epigenetic modifications have been also documented. The direct action of UV and IR over DNA and RNA produces abnormal covalent interactions between adjacent molecules. Dietary supplementation with antioxidants, such as *tert*-butyl hydroquinone, seleno-L-methionine, vitamin C, vitamin E, α-lipoic acid, and *N*-acetylcysteine (NAC), helps to mitigate epigenetic changes. Some plants such as raspberries, blueberries, strawberries, and grapes exhibit antioxidant activities related to a high content of polyphenolic compounds, which also stimulate the synthesis of endogenous antioxidants such as glutathione (GSH). Together they represent a good dietary strategy to prevent oxidative damage to macromolecules when UV or IR exposure occurs

(Alcaraz et al. 2013; Almeida et al. 2013; Chen et al. 2013; Xu et al. 2013), can protect normal tissue during radiotherapy. Plant compounds containing polyphenolic compounds, such as raspberries, blueberries, strawberries, and grapes, exhibit antioxidant activities against IR (Fig. 8.1).



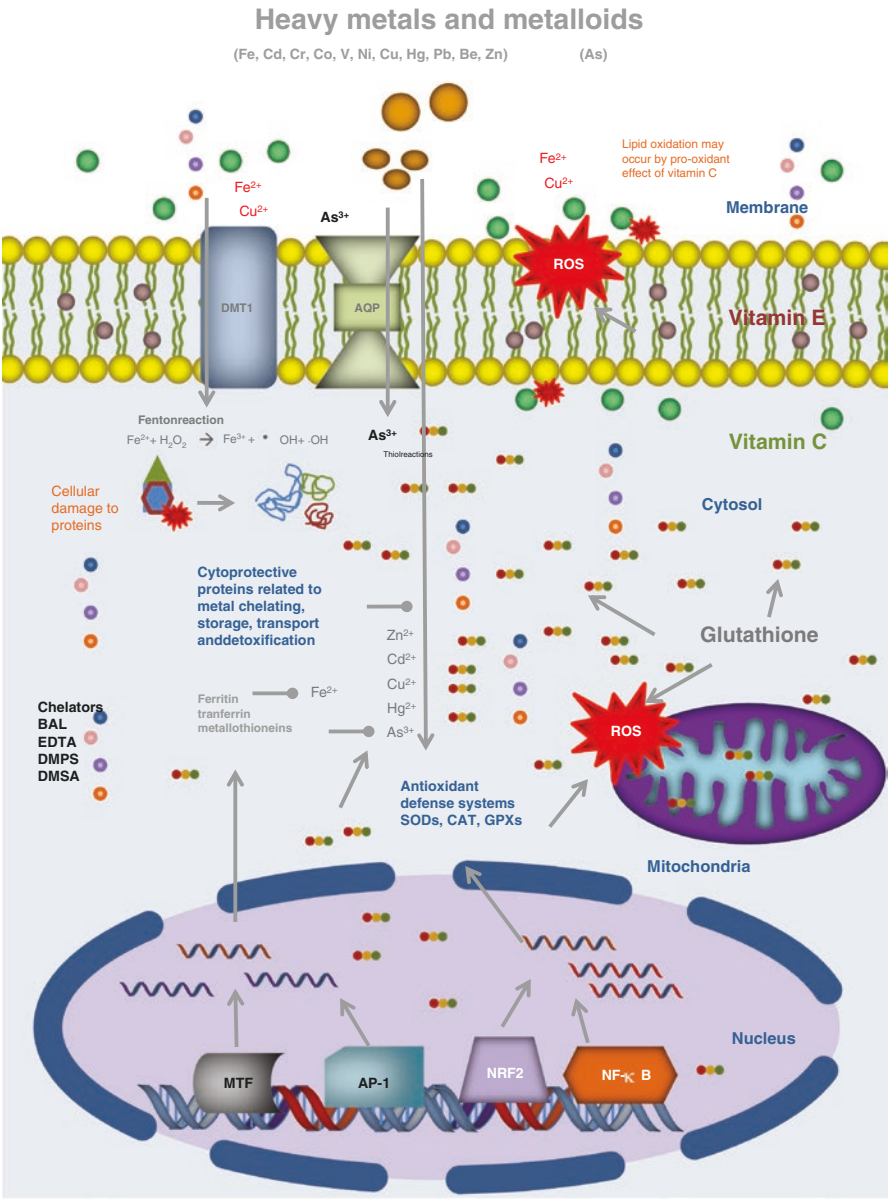
8.4.2 Heavy Metals

Heavy metals are among the most important pollutants in the environment. Exposure to heavy metals occurs not only through the diet but also through groundwater contamination, mining, leather tanning, metal plating, and iron and steel founding. Metals or metalloids, such as arsenic, beryllium, cadmium, chromium, and nickel, are considered as carcinogens (International Agency for Research in Cancer 2012). These metals, as well as cobalt, lead, mercury, and vanadium, are biologically active and toxic even at low levels of exposure. Since metals are elements characterized by their tendency to lose electrons, they are thought to be toxic by virtue of ROS generation. Gestational exposure to metals with negative effects in children growth and development has been documented in several epidemiological studies (Gardner 2012; Harari et al. 2012; Kippler et al. 2012a, b, c). Metals can interact with each other and enhance or reduce toxicity. For example, chronic arsenic exposure alters iron metabolism (Hernández-Zavala et al. 1999), while selenium facilitates the excretion of arsenic in the bile (George et al. 2013). However, the epidemiological evidence needs to be elucidated in experimental models. Acute poisoning is usually treated using chelation therapy that speeds elimination from the body. Chelating agents for the treatment of acute arsenic, mercury, and lead poisoning include British anti-Lewisite (BAL), 2,3-dimercapto-1-propanesulfonic acid (DMPS), and dimercaptosuccinic acid or succimer (DMSA) (Cao et al. 2015). Ethylenediaminetetraacetic acid (EDTA) is used to chelate several toxic metals, including lead (Fig. 8.2).

Fig. 8.2 The coordinated action of antioxidants and chelators in the control of cellular toxicity caused by heavy metals and metalloids. Exposure to heavy metals, such as iron (Fe), chromium (Cr), cadmium (Cd), cobalt (Co), vanadium (V), nickel (Ni), copper (Cu), lead (Pb), mercury (Hg), beryllium (Be), zinc (Zn), and metalloid arsenic (As), may occur via environmental pollution from natural or anthropogenic sources. They might be present in the air, soil, water, and also in food or drugs. Some metals such as Fe^{2+}, Cd^{2+}, Zn^{2+}, and Cu^{2+} enter the cell via divalent metal transporter 1 (DMT1). Members of the aquaporins (AQP) subfamily 3, 7, and 9 may transport As^{3+} into mammalian cells. ROS generation and oxidative stress constitute the main mechanism of toxicity through chemical reactions with endogenous peroxides and metals as in the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH$). Initial damage may involve the membrane oxidation through lipid peroxidation. Supplementation with vitamin E protects against membrane lipid peroxidation. Oxidative damage to proteins also occurs during metal exposure. Coordinated actions of intracellular antioxidant systems such as reduced glutathione (GSH) and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), among others help to control oxidative stress. Proteins such as ferritin, transferrin, and metallothioneins are regulators of intracellular levels of heavy metals and metalloids. The expression of antioxidant systems and protein-related heavy metal sequestration involves the nuclear translocation of transcription factors, i.e., nuclear factor (erythroid-derived 2)-like 2 (Nrf2), nuclear factor kappa B (NF-κB), the metal-responsive transcription factor-1 (MTF-1), and activator protein 1 (AP-1), respectively. Exogenous chelators such as dimercaprol (also named as British anti-Lewisite (BAL)), ethylenediaminetetraacetic acid (EDTA), 2,3-Dimercapto-1-propanesulfonic acid (DMPS), and dimercaptosuccinic acid (DMSA) are used to treat metal intoxication. Under specific circumstances, vitamin C or E may be prooxidants in the presence of heavy metals (see text for details)

8.4.3 *Pesticides*

In spite of the fact that the use of pesticides in agriculture is regulated in many countries belonging to the Organization for Economic Cooperation and Development (OECD 1981), the intensive use of pesticides (insecticides, fungicides, herbicides, rodenticides,



and fumigants) has become a major and serious environmental and human health problem. Many pesticides induce oxidative stress in animal models (Tomita et al. 2005; Ray et al. 2007; Dinis-Oliveira et al. 2008). There is evidence that exposure to pesticides contributes to chronic diseases such as cancers; diabetes; neurodegenerative disorders, like Parkinson, Alzheimer, and amyotrophic lateral sclerosis; prenatal development; birth defects; and reproductive disorders (Mostafalou and Abdollahi 2013). Epidemiological and experimental studies have implicated the fungicide maneb, the herbicide paraquat, or the insecticide rotenone in Parkinson (Tanner et al. 2011; Singhal et al. 2013; Goldman 2014). Sylmarin (3,5,7-trihydroxy-2-[2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-2,3-dihy-dro-1,4-benzodioxin-7-yl]-2,3-dihydrochromen-4-one) and melatonin (*N*-acetyl-5-methoxytryptamine) reduce the oxidative damage of maneb, paraquat, and diquat (Xu et al. 2007; Singhal et al. 2013).

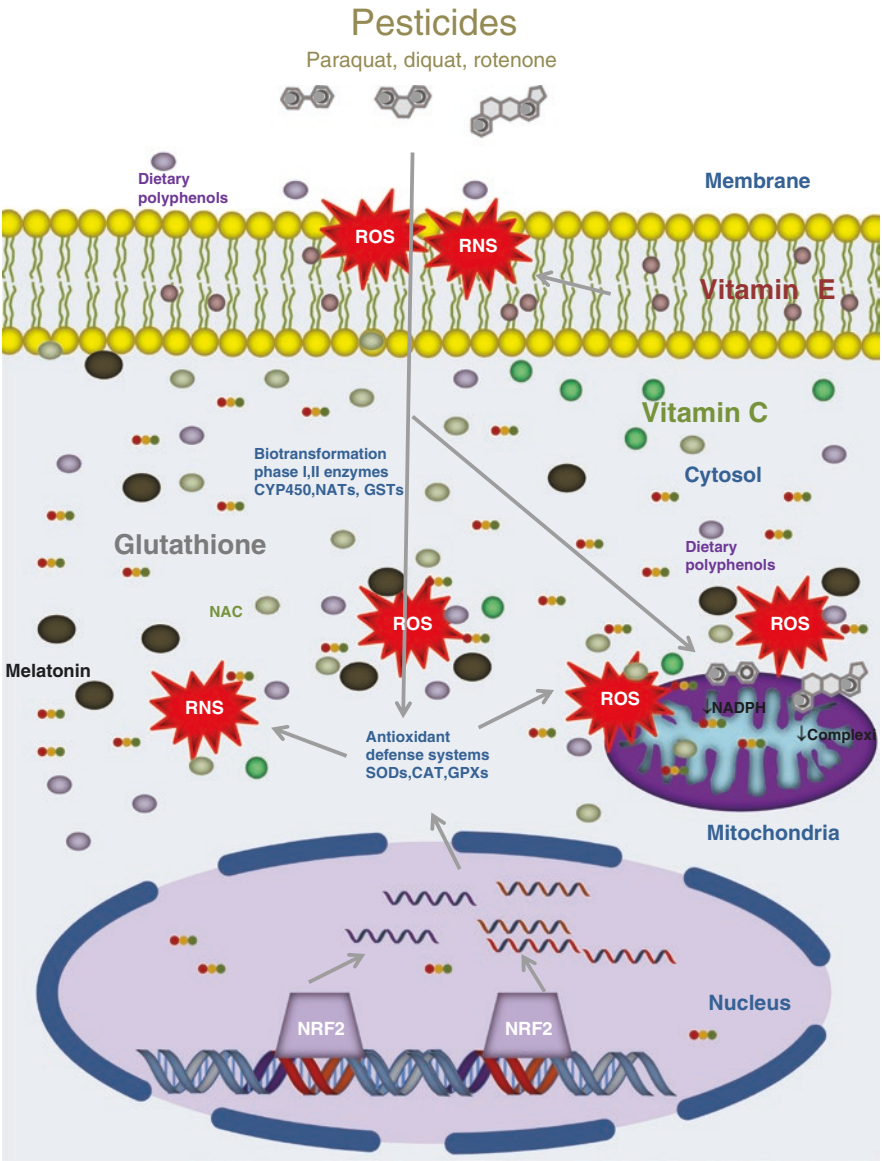
Neurotoxicity of the pesticides, rotenone and paraquat, is associated with disturbance of mitochondrial bioenergetic function, reactive oxygen metabolism, and redox function that promote α-synuclein aggregation (Thany et al. 2013). Several pesticides have displayed carcinogenic potential in experimental models (Environmental Protection Agency 2012), as well as in humans, mainly, pesticide manufacturing workers (Acquavella et al. 1996; Leet et al. 1996; Fryzek et al. 1997; Kogevinas et al. 1997), farmers (Blair and Freeman 2009), and agricultural workers (Rastogi et al. 2009). Stimulation of ROS production and disturbance of the total antioxidant capability are among the mechanisms by which most pesticides induce toxicity (Abdollahi et al. 2004) (Fig. 8.3).

8.4.4 Air Pollution

Air pollution comprises a complex mixture of individual chemical constituents, which vary around the globe due to differences in the sources of pollution, climate, and meteorology, but the mixtures of ambient air pollution invariably contain Group

Fig. 8.3 Dietary polyphenols and endogenous antioxidants protect against oxidative stress induced by exposure to pesticides. Environmental exposure to pesticides such as paraquat, diquat, and rotenone leads to the intracellular generation of reactive species from reactive oxygen species (ROS) and reactive nitrogen species (RNS). Initially, ROS and RNS oxidize cellular structures such as membranes which result in lipid peroxidation which can be prevented by vitamin E. Rotenone acts as direct inhibitor of complex I of respiratory chain in mitochondria, whereas paraquat and diquat produce a nonselective inhibition of respiratory complex together with ROS formation leading to mitochondrial dysfunction. Endogenous antioxidant defense systems such as reduced glutathione (GSH), glutathione peroxidase (GPX), catalase (CAT), cytosolic and mitochondrial superoxide dismutase (SOD2) participate in the control of oxidative stress caused by ROS after pesticide exposure. Dietary polyphenols might help indirectly to increase endogenous antioxidant capacity by modulation of antioxidant gene expression. Part of the mechanism of regulation involves the activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), one of the most important regulators of cellular antioxidant systems. Supplementation with vitamin C and administration of *N*-acetylcysteine (NAC) ameliorate the toxicity of pesticides

1 human carcinogens (Straif et al. 2013). Principal air pollutants termed "criteria" pollutants by the US EPA include carbon monoxide (CO), lead, nitrogen dioxide (NO_2), particulate matter (PM) in two size ranges ($PM_{2.5}$ and PM_{10}), ozone (O_3), and sulfur dioxide (SO_2) (United States Environmental Protection Agency 2010). Air pollution increases the risk of respiratory, neurological, and heart diseases, as well as developmental disorders (Loomis et al. 2014; Curtis et al. 2006; MohanKumar



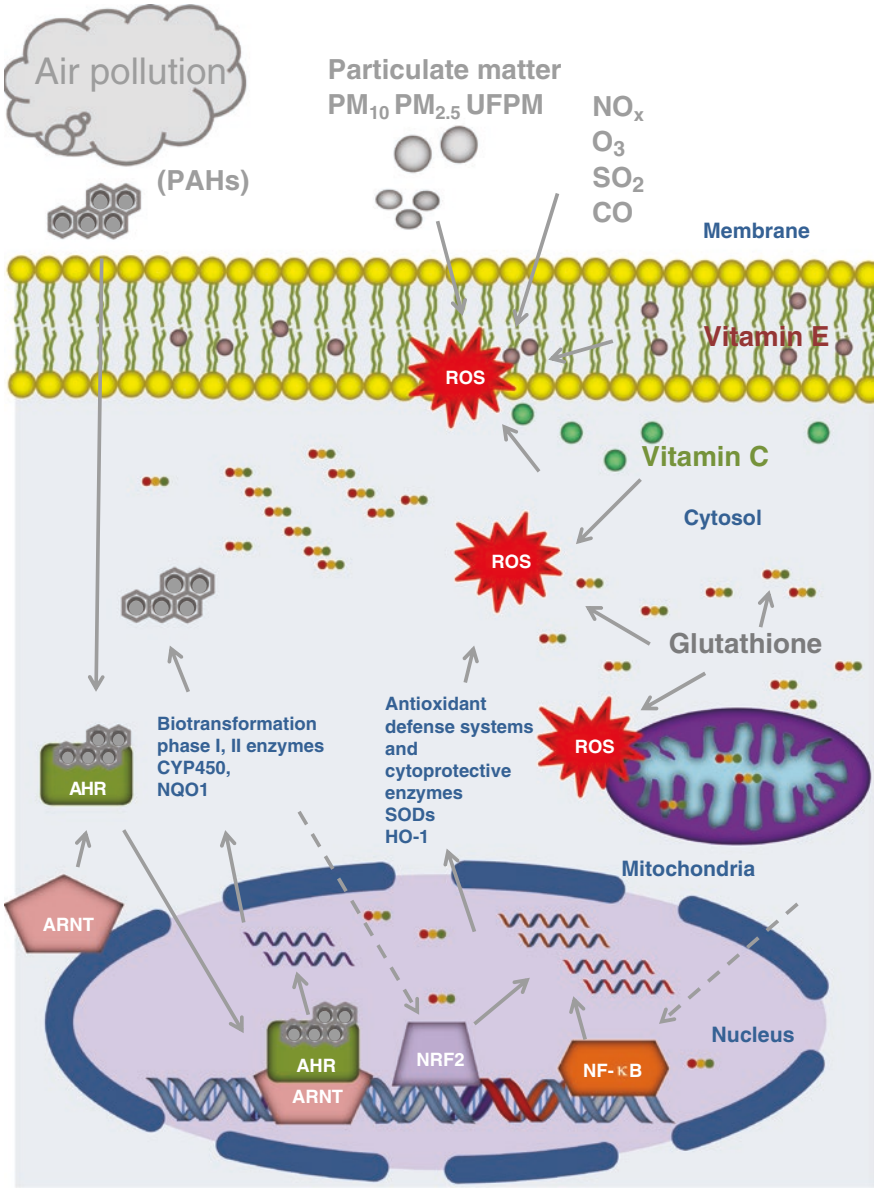
et al. 2008; Block and Calderón-Garcidueñas 2009; Yang and Omaye 2009; Anderson et al. 2012; Loane et al. 2013; Fleischer et al. 2014). PM, O_3, SO_2, NO_2, CO, and PAH induce oxidative stress either directly or indirectly via their by-products (Hassing et al. 2009). Moreover, deficient antioxidant responses due to genetic polymorphisms in glutathione *S*-transferase mu 1 (GST M1) and heme oxygenase 1 (HO-1) induced greater morbidity following exposure to PM air pollution than in wild-type subjects (Limón-Pacheco and Gonsebatt 2009). Positive associations between lipid peroxidation and air pollution, as well as between protein oxidation and PM, have been observed in populations exposed to air pollution (Patel et al. 2013; Rossner et al. 2013). Exposure to PM from burning biomass fuels in rural communities is associated with reduced antioxidant defenses, oxidative stress, inflammation of the airways, and pulmonary dysfunction (Oluwole et al. 2013; Mukherjee et al. 2013).

In vitro and in vivo investigations, as well as epidemiological studies (Brunekreef and Holgate 2002), suggest that the adverse effects of exposure to air pollution are strongly related to oxidative stress-associated reductions in redox capacity (GSH/GSSG ratio); increases in lipid and protein oxidation (Marchini et al. 2014); increases in high-sensitivity C-reactive protein (hs-CRP), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and plasminogen activator fibrinogen inhibitor-1 (PAI-1) (Chuang et al. 2007); and chronic inflammation (Rückerl et al. 2007; Calderón-Garcidueñas et al. 2013; Marchini et al. 2014). In animal models, pulmonary and gastrointestinal tract exposures to PM are associated with oxidative DNA damage (Møller et al. 2013). In vitro studies suggest that the number of particles may be more effective in inducing oxidative stress during these conditions than the particle components (Kroll et al. 2013). Therefore, the formation of ROS and oxidative damage following exposure to air pollutants has been suggested to be an important underlying mechanism of air pollution toxicity (Fig. 8.4).

To attenuate the adverse effects of air pollutants on health, the use of antioxidants has been explored. For example, in a study of short-term exposure to O_3, healthy adults received 800 or 1600 IU of vitamin E for a minimum of 9 weeks, and the

Fig. 8.4 Cellular response to air pollution involves the coordinated action of biotransformation enzymes and antioxidant defense systems. Principal air pollutants termed "criteria" pollutants include carbon monoxide (CO), nitrogen dioxide (NO_2), particulate matter (PM) in two size ranges ($PM_{2.5}$ and PM_{10}), ozone (O_3), and sulfur dioxide (SO_2). PM, O_3, SO_2, NO_2, and CO induce oxidative stress either directly or indirectly via their by-products. PM comprises a complex mixture of individual chemical constituents which includes polycyclic aromatic hydrocarbons (PAHs). Some of the PAHs present in air pollution are considered human carcinogens. Antioxidant defense systems help to control ROS production. PAHs modulate the expression of genes related to biotransformation enzymes such as cytochromes P450 via the aryl hydrocarbon receptor (AHR)/aryl hydrocarbon receptor nuclear translocator (ARNT). The PAH-AHR/ARNT complex initiates the transcription of genes with xenobiotic responsive element (XRE) sequences. The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor-kappa B (NF-κB) modulate antioxidant genes. Glutathione (GSH) has a dual effect as antioxidant and as a substrate for conjugation reactions helping in the inactivation and elimination of bioactive metabolites of PAHs. Vitamin E and C have protective effects against air pollutants such as O_3 (see text for details)

results revealed no significant differences between the responses in the supplemented and placebo-treated groups in parameters such as hematocrit and hemoglobin values, glutathione concentrations in red blood cells, and enzymes, such as acetyl cholinesterase, glucose-6-phosphate dehydrogenase, and lactic acid dehydrogenase (Posin et al. 1979; Hackney et al. 1981). The protective effects of vitamins E and C and β-carotene supplementation have been studied in human populations



exposed to air pollutants, such as O_3 (Grievink et al. 1998, 1999a, b; Romieu et al. 1998, 2002). For example, 15 mg of β-carotene, 75 mg of vitamin E, and 650 mg of vitamin C were administered once daily for 3 months to amateur cyclists, which attenuated the effects of exposure to ozone (Grievink et al. 1998). In another study, 100 mg of vitamin E and 500 mg of vitamin C daily for 15 weeks provided partial protection against the acute effects of ozone (Grievink et al. 1999a, b). In asthmatic children, beneficial effects were also observed with 50 mg/day of vitamin E and 250 mg/day of vitamin C (Romieu et al. 2002). In contrast, in subjects who were sensitive to O_3, supplementation with vitamins C (500 mg/day) and E (100 mg/day) demonstrated no protective effects (Mudway et al. 2006).

8.5 Antioxidant Therapy: Scope and Limitations

The role of nutrition and dietary antioxidants as potential preventive and treatment therapies against diseases associated with exposure to multiple pollutants is a key objective in the current context of our environment. An inverse correlation between vegetable consumption and disease prevention and/or treatment, such as cancer incidence, has been reported in both animal and human studies, indicating that the success of whole foods or mixtures is stronger than that obtained for the use of plant isolates. Plant foods are chemically complex and have become a source of essential nutrients with antioxidant activities (Thompson et al. 1999). In developing countries, approximately 60% of cancers of the oral cavity, pharynx, and esophagus are thought to result from micronutrient deficiencies that are related to a restricted diet that is low in fruits and vegetables. Some evidence suggests that a very low intake of fruits and vegetables is related to increases in risk compared with higher intake; however, few benefits exist if we do not take the dose-response relationship into consideration (Key et al. 2004). Although the overall effectiveness of antioxidants in disease prevention can be established only through randomized controlled trials, differences in dietary composition, such as vitamin and micronutrient intake, may contribute to design flaws and lead to null findings (Morris and Tangney 2011). The success of antioxidant therapy varies based on several aspects, some of which are discussed below.

8.5.1 Nutritional Status and Dietary Antioxidant Composition

Although several studies have suggested that dietary antioxidants have positive effects on human health, several considerations must be taken into account. For example, prior to implementing antioxidant therapy, adequate nutrition in the target population must be achieved, as it is fundamental for health status. Therefore, it is necessary to estimate nutrient intake based on food consumption, as well as the antioxidant composition of the foods consumed (Buttriss and Benelam 2010; Elmadfa and Meyer 2010), because this is important to identify the chemical forms

of the antioxidants, their concentrations, and the potential chemical interactions that may impact their bioavailability. For example, given the diversity of the chemical structures of some dietary antioxidants, such as polyphenols (Pérez-Jiménez et al. 2010), and the scarce information available regarding mixture composition, it is difficult to estimate the total specific content of each antioxidant molecule in various foods. In addition, regional soil quality, meteorological aspects, and differences in plant varieties as sources of natural antioxidants may influence antioxidant content, which could limit comparisons of the effectiveness of dietary antioxidant therapies between countries (Elmadfa and Meyer 2010). Hence, statements regarding potential therapeutic use should be made cautiously.

8.5.2 Presence of Pollutants in Antioxidant-Containing Foods

Another important aspect to consider is the potential hazards related to the presence of toxic products or contaminants, which may adversely affect health. Among these are substances formed during food preservation, such as nitrosamines, or those that form during cooking, such as polycyclic aromatic hydrocarbons (PAH) and heterocyclic amines (HA) (Jakszyn et al. 2004). Drugs, such as chemotherapeutic agents (Conklin 2000), and industrial pollutants, such as agrochemicals and pesticides, may also enter the food chain. Although pesticide residues on crops and food products are monitored through the use of maximum residue limits (MRL), detectable residues at or below MRLs may be found in vegetables and fruits, increasing the likelihood that they will enter the human food chain (Ripley et al. 2000; Waliszewski et al. 2008; Aldana-Madrid et al. 2011; Bakırcı et al. 2014), which is important because the increased consumption of natural sources of antioxidants, such as vegetables and fruits, may lead to increases in health risks associated with low but chronic daily exposures to those pesticides, as has been recently demonstrated in the case of pyrethroid pesticide exposure in children (Waliszewski et al. 2008; Lu et al. 2010; Aldana-Madrid et al. 2011; Bakırcı et al. 2014). Humans are exposed to numerous and complex mixtures of chemicals in the diet that may cause and/or modulate disease outcome. In human, an estimated 24% of diseases are caused by exposure to environmental pollutants (Hou et al. 2012). Carcinogens, such as PAH, aflatoxin B (AFB), *N*-nitroso compounds, heterocyclic amines, and acrylamide, can be found in foods. Some of these carcinogens are not naturally occurring but originate during preservation or cooking (Jakszyn et al. 2004).

8.5.3 Bioavailability of Dietary Antioxidants

Controversial results have been obtained in intervention studies intended to diminish the mortality or morbidity of diseases associated with chronic oxidative stress. From our perspective, this controversy emphasizes the lack of information

regarding the bioavailability of food antioxidants in human and animal subjects, as well as the role of genetic polymorphisms in the absorption, metabolism, and accumulation of antioxidants. Do bioavailable antioxidants preferentially accumulate in organs, similarly to what occurs with some xenobiotics? For example, the half-life of α-tocopherol in the brain is one or two orders of magnitude longer than in any other organ (Ulatowski and Manor 2013). We also need to document how exogenous antioxidants are incorporated into the cell and the impact of genetic polymorphisms on the absorption, metabolism, and accumulation of antioxidants. Several genetic variants of sodium-dependent vitamin C transporters (SLC23A1 and SLC23A2) that affect vitamin C status have been characterized in human populations (Michels et al. 2013). Are other membrane transporters involved in the cellular intake of water-soluble antioxidants? Do intracellular receptors for lipid- or water-soluble antioxidants exist? This information would help us understand the controversial results of some epidemiological studies and design new nutritional therapeutic studies that take these important factors into consideration.

Flavonoids, as well as carotenoids, polyphenols, alkaloids, nitrogen compounds, and organosulfur (Liu 2004), are present in fruits, vegetables, and plant-derived beverages that modulate phase I and II enzyme activity, resulting in the decreased carcinogenicity of xenobiotics in both in vitro and in vivo models (Moon et al. 2006). These effects most likely result from the downregulation or inhibition of cytochromes P450 (CYP) enzymes that affect the bioactivation of carcinogens and the ability of polyphenols to quench free radicals or directly activate nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (Balogun et al. 2003; Kang et al. 2007). However, observations made in vitro or in animal models cannot always predict the effects that will be observed in humans due to differences in absorption, bioavailability, CYP polymorphisms, gender differences, or diet composition (Moon et al. 2006; Lee et al. 2011).

8.5.4 Antioxidant Capacity In Vitro Versus Antioxidant Efficacy In Vivo

In the last years, synthetic and natural compounds are evaluated for their antioxidant profile, using a number of assays which are focused on the effects of delaying or preventing oxidative stress, in most cases without further validation or with lack of substrate specificity. The most used assays to test antioxidant capacity in biological and food samples are Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), total radical-trapping parameter (TRAP), and ferric reducing ability of plasma (FRAP) (Antolovich et al. 2002; Huang et al. 2005; Prior et al. 2005; Hermans et al. 2007; Bast and Haenen 2013). The main problem present in biological samples is the complexity of antioxidants present. All organisms have a battery of antioxidant systems that may be not properly evaluated by these in vitro assays due to the unique biochemical profile of specific antioxidants. In the best case, antioxidant capacity tests will indicate the overall activity of several

antioxidants at the same time, but it is clear that standardized procedures should be used. A good antioxidant capacity evaluation must include different assays to characterize antioxidant activity in vitro and in vivo. In vivo models are still necessary to understand the toxicokinetics (absorption, distribution, metabolism, and elimination) of antioxidants. Also, in vitro antioxidant assays are relevant for primary evaluation of antioxidant profile in large samples, such as plant extracts. However, a positive antioxidant capacity of compounds evaluated in vitro should be taken with caution and not as a cure or panacea against oxidative damage. The multivariate actions of antioxidants provided by dietary and pharmacological therapies suggest a contribution through increasing the ability to maintain homeostasis by increasing metabolic capacity fundamentally. Thus, most pharmacological activities studied in vitro remain still inconclusive when tested in vivo mostly due to the fact that bioavailability, biotransformation, and bioactivation are not always taken into consideration.

Table 8.1 Aspects to consider in the antioxidant therapy against environmental induced oxidative stress

Consideration	Scope	Limitation
Nutritional status and dietary antioxidant composition	Well nourished individual Balanced diet Enriched diet with antioxidants	Bioavailability, chemical composition, chemical interactions of antioxidants in food Access to good quality food Fresh food availability
Toxic products and pollutants in antioxidant-containing foods	Food free of preservatives, agrochemicals and pesticides Adequate storage, preparation and cooking	Production of food depends on industrialized processes which might introduce toxic chemicals in the food chain Formation of undesirable toxic compounds during food storage and preparation, lost of antioxidant properties
Bioavailability of therapeutic antioxidants	Suitable absortion, transport and distribution of antioxidants in the body Chemical stability	Genetic polymorphisms in the absorption, metabolism and accumulation of antioxidants restrict their protective effect
Interactions between environment, diet, lifestyle and antioxidant therapy	Requirements of antioxidants according to lifestyle habits and physical activity Environment free of chronic oxidative stress inducers	Studies needed to assess diet interaction, environment and antioxidant therapies Pollution (inducing oxidative stress in the environment) present everywhere
Antioxidant capacity in vitro versus antioxidant efficacy in vivo	The in vitro tests reveals the potential use of a compound as antioxidant	Further validation of the in vitro assays to test antioxidant capacity is needed In vitro and in vivo assays to test toxicodynamics and toxicokinetics Bioavailability, biotransformation and bioactivation are not always taken into consideration in vitro

8.6 Conclusions

Although antioxidant therapy has demonstrated effectiveness in the prevention and treatment of different oxidative pathologies, it is clear that the protective effects cannot be generalized as a "panacea" for all diseases. The utility of antioxidant therapies to protect organs and tissues of biological systems against environmental pollutant-induced oxidative damage is encouraging, but not conclusive. In most cases, the benefits obtained by supplementation of antioxidants in diet are due to the ability of these molecules to maintain intracellular homeostasis for a preventive action, rather than corrective one. Moving from in vitro to in vivo testing models requires consideration of a number of factors that influence the therapeutic outcome, mainly related to the physicochemical antioxidant and metabolic interaction properties within organs and tissues (Table 8.1). These considerations could lead to a progress in the design of valuable and effective antioxidant therapies complementary to baseline therapy for specific organ diseases associated with oxidative stress due to exposure to environmental pollutants.

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Chapter 9
Antioxidant Therapy Against Persistent Organic Pollutants and Associated Diseases

Bernhard Hennig, Michael C. Petriello, Bradley J. Newsome, Jordan T. Perkins, and Dandan Liu

Abstract Environmental pollutants are recognized as modulators of metabolic disorders or noncommunicable diseases such as cancer, cardiovascular disease, diabetes, and obesity. Nutritional interventions have emerged as means to prevent the development of metabolic diseases and to modulate cellular mechanisms responsible for pro- and antioxidant responses, inflammatory signaling cascades, and environmental toxicant clearance. We review the role of persistent organic pollutants (POPs) in disease development along with the underlying molecular mechanisms controlling the development of oxidative stress-sensitive and/or inflammatory diseases ranging from metabolic syndrome-related disorders to cancer. The use of antioxidant therapies to mitigate the development of environmental pollutant-induced diseases is discussed as the basis of a potent public health intervention strategy. While nutritional interventions have long been seen as vital components for decreasing complications associated with metabolic diseases, they are emerging as realistic, cost-effective means to decrease adverse effects of environmental POPs on health outcome. This chapter also reviews common mechanisms of toxicity of emerging organic contaminants with a focus on model toxicants such as POPs and how antioxidant therapies may be used as sensible means of modulating POP-induced

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oxidative stress and chronic inflammation that lead to disease risks. Diets rich in antioxidants may contribute to cellular protection against POP-induced inflammatory reaction by upregulating antioxidant cellular defenses, increasing excretion rates of POPs, and/or downregulating pro-inflammatory signaling cascades.

Keywords Environmental persistent organic pollutants • Reactive oxygen species • Oxidative stress • Inflammation • Diseases • Dietary antioxidants

9.1 Introduction

Exposures to human-made environmental pollutants occur daily in occupational, educational, and at-home settings. An extensive number of legacy and emerging contaminants can be found in nearly every terrestrial and aquatic ecosystem. While thousands of these compounds have yet to be thoroughly tested to determine their human and environmental risk potentials, many of these contaminants, including persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and organochlorine (OC) pesticides, share structural and functional commonalities with well-understood model toxicants. POPs represent a group of organic contaminants produced initially as pesticides and industrial chemicals that are highly resistant to biological, chemical, and photolytic degradation. These pollutants accumulated in soil and water may transfer to organs and tissues of biological systems through the food chains and cause adverse health effects on human or biological effects on soil fauna and flora (Chen et al. 2005; Yu et al. 2010; Maliszewska-Kordybach et al. 2013; Fernandez et al. 2014). Environmental pollutants can alter or disrupt multiple organ systems, cell types, and signaling pathways through general mechanisms such as increasing cellular oxidative stress (Limón-Pacheco and Gonsebatt 2009). Laboratory and epidemiological studies link exposures to pollutants to chronic pathologies such as cancers, liver diseases, diabetes, obesity, and cardiovascular disease.

Fortunately, functional plant components in human diets have significant antioxidant activity and anti-inflammatory actions and so serve as the basis for therapeutic or preventative nutritional strategies to counteract pollutant-induced oxidative stress and diseases (Csiszar et al. 2006; García-Lafuente et al. 2009; Pandey and Rizvi 2009). Much attention has been directed on pollutant remediation to reduce potential adverse health effects associated with pollutant exposure. However, these approaches are extremely time-consuming and expensive and do not address effects linked to prior exposures. Therefore, it is important to identify and implement easy-to-translate nutritional therapies, which can effectively prevent pollutant-induced disease.

The role of a healthy food in preventing chronic noncommunicable diseases, such as heart disease, diabetes, cancers, and degenerative diseases, is now well

accepted (Abuajah et al. 2015) but primarily in response to historically relevant risk factors such as gender, genealogy, and unhealthy lifestyle behaviors, mainly smoking, alcohol consumption, and malnutrition. As more data now implicate exposures to environmental pollutants as an emerging risk factor for noncommunicable diseases, nutritional intervention may prove to be an effective strategy for preventing pollutant-induced diseases. Plant-derived antioxidants, mainly fruit and vegetable polyphenolic compounds, thought to provide health benefits by directly scavenging and eliminating superoxide radicals ($^{\bullet}O_2^-$) and related highly reactive and toxic reactive oxygen species (ROS) or by upregulating ROS-scavenging antioxidant enzymes. Therefore, diets rich in polyphenols, such as epigallocatechin gallate (EGCG), quercetin, and curcumin, prevent oxidative stress and decrease human body burdens of environmental pollutants, ultimately protecting against toxicant-induced noncommunicable diseases.

In this chapter, we will describe common mechanisms of environmental pollutant toxicity with a focus on model toxicants such as POPs and how antioxidant therapies may prove to be sensible means of preventing disease risks. The following major points will be discussed:

1. Mechanisms of POP toxicity
2. Epidemiological links between environmental pollutants and noncommunicable diseases
3. Use of antioxidant therapies for reducing inflammation and associated diseases
4. Use of antioxidant therapies specifically to reduce risks associated with POP-induced noncommunicable diseases
5. Potential mechanisms of antioxidant protection against POP-induced toxicity

9.2 Persistent Organic Pollutants (POPs) and Mechanisms of Toxicity

POPs have been linked to a multitude of negative health effects linked to various types of cancer, cardiovascular disease, hormone disruption, diabetes, and obesity (Everett et al. 2011; Malarvannan et al. 2013; Sjöberg Lind et al. 2013; Arrebola et al. 2014; Reaves et al. 2015). The Stockholm Convention on Persistent Organic Pollutants held in 2001 sought to address the chemical toxicities associated with the "dirty dozen," 12 POPs associated with significant public health risks, by placing a global ban on their use (US EPA 2014). This initial list of banned POPs has now been extended to include various flame retardants, organometallic compounds, and polycyclic aromatic hydrocarbons, with a strong emphasis placed not only on environmental remediation efforts but also on further understanding modes of toxicity and developing potential health intervention strategies.

Among the growing list of POPs, dioxins and dioxin-like substances are of major health concern due to their highly toxic potential. "Dioxins and dioxin-like substances" commonly refer to PCDDs, PCDFs, and PCBs. These compounds possess

similar toxicological profiles and common mechanisms of toxicity (Faroon et al. 2003; Canady et al. 2010). POPs tend to bioaccumulate and bioconcentrate through the food chain primarily due to their highly stable, lipophilic chemical structures, thus enabling potential human exposure at toxicologically relevant concentrations even though exposures may initially appear to be discrete and limited (Ritter et al. 1995). Many POPs induce oxidative stress and chronic inflammation that subsequently lead to disease risks. However, all POPs activate their pro-inflammatory signaling cascades via identical mechanisms. As such, mechanisms of toxicity are described for two major POP categories: dioxin-like and non-dioxin-like pollutants. This delineation is critical because dioxin-like chemicals produce toxicity primarily through aryl hydrocarbon receptor (AhR)-mediated events, whereas non-dioxin-like chemicals may produce toxicity via endocrine disruption and other alternative mechanisms (Fig. 9.1).

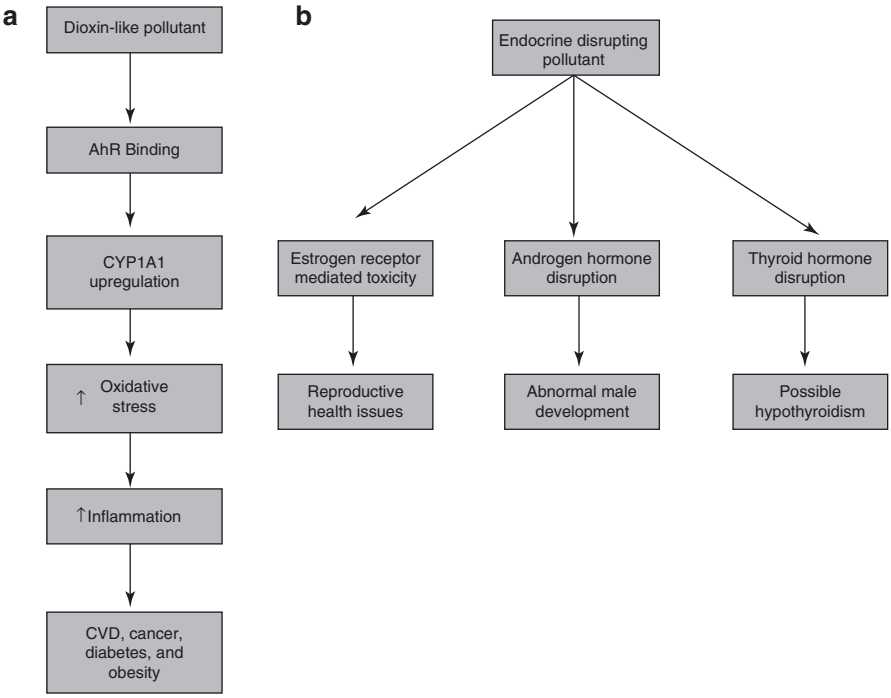

Fig. 9.1 POP toxicity is mediated through both (**a**) aryl hydrocarbon receptor (AhR)-dependent and (**b**) AhR-independent toxicity pathways. (**a**) Dioxin-like pollutants, such as coplanar PCBs, bind the AhR and upregulate detoxification-regulated genes such as CYP1A1. CYP1A1 activation leads to the formation of superoxide and related ROS, which can lead to chronic inflammation in multiple organ/tissue types. (**b**) Endocrine-disrupting chemicals, such as certain pesticides, can lead to hormone and reproductive toxicities by mimicking endogenous compounds or disrupting hormonal control mechanisms in different organs. Antioxidants and particular polyphenols can be used to downregulate POP-induced inflammatory pathways and associated diseases triggered by environmental pollutants

9.2.1 Dioxin-Like Pollutants

Approximately 30 dioxin-like compounds are considered to pose significant human toxicity, while 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) possesses the highest toxicity of this group of compounds and serves as the benchmark for dioxin-like pollutant toxicity equivalence profiles for the remaining pollutants (Van den Berg et al. 2006). TCDD induces a series of toxicological effects, including teratogenesis, immunosuppression, tumor promotion, and inflammatory-related diseases (Mimura and Fujii-Kuriyama 2003). The toxic effects of dioxin-like chemicals are primarily mediated by AhR, which is a widely expressed nuclear transcription factor that binds a broad range of xenobiotics, with especially high binding affinity for TCDD (Fernandez-Salguero et al. 1997; Mimura et al. 1997; Shimizu et al. 2000; Hankinson 2005). Ligand-activated AhR translocates from cellular cytoplasm into the nucleus where it binds AhR translocator (Arnt), forming an AhR/Arnt heterodimer. The heterodimer then binds to the xenobiotic response element (XRE) in the promoter region of its target genes such as the cytochrome P450 family gene P4501A1 (CYP1A1) and induces transcription (Ko et al. 1996; Kobayashi et al. 1996). The expression of these target genes further activates downstream reactions that expand the toxic effects of TCDD.

The production ROS is considered as one of the major features underlying TCDD-mediated AhR activation and is believed to be a key determinant of TCDD-induced toxicity (Mates et al. 2010). Coplanar PCBs also act as dioxin-like pollutants. The toxicities of PCB variants, called congeners, of which there are 209, depend upon both the number and position of chlorine atoms bound to a biphenyl structure; greater toxicity is generally associated with a higher number of chlorines (Bruner-Tran and Osteen 2010). Additionally, non-ortho PCBs, commonly referred to as coplanar PCBs, that lack chlorine substitutions at the ortho positions of both phenyl rings are generally more toxic than ortho-substituted PCB congeners (Giesy et al. 2000). Coplanar PCBs share structural and physiological similarities to TCDD and as such exert their toxic health effects through activation of AhR (Kafafi et al. 1993; Okey et al. 1994). The mechanism of coplanar PCB-induced toxicity is a combination of high specificity for AhR binding, persistent AhR activation, and an increase in ROS production through CYP1A1-mediated uncoupling (Lim et al. 2008). It is interesting to note that ROS contribute to the induction of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione *S*-transferases (GST), thioredoxins (TRX), and thioredoxin reductases (TRXR) (Mann et al. 2007; Myers 2012).

Depending on the level of cellular ROS, distinct redox-sensitive transcription factors are activated and trigger different biological and biochemical responses. A low level of ROS induces NF-E2-related factor 2 (Nrf2), a transcription factor implicated in the upregulation of genes coding for antioxidant enzymes (Gloire et al. 2006). A moderate level of ROS induces an inflammatory response mediated by the activation of activator protein-1 and nuclear factor kappa B (NF-κB), the first transcription factor shown to be redox regulated (Legrand-Poels et al. 1990; Schreck

et al. 1991; Gloire et al. 2006). High levels of ROS lead to perturbation of the mitochondrial permeability transition pore and disruption of the electron transport chain, resulting in apoptosis or necrosis (Gloire et al. 2006). Nuclear factor-kappa B (NF-κB) plays a crucial role in regulating immune response and inflammation (Hayden and Ghosh 2004). In the absence of cellular signals, NF-κB is sequestered in the cytoplasm by association with IκB. Upon stimulation, IκB is rapidly phosphorylated and degraded by the proteasome, which releases NF-κB that then translocates into the nucleus where it activates the transcription of target genes (Bonizzi and Karin 2004; Hayden and Ghosh 2004). Oxidant-induced NF-κB activation using hydrogen peroxide (H_2O_2) is highly cell-type specific and involves quite different mechanisms (Li and Karin 1999). For example, early studies revealed an atypical mechanism of NF-κB activation resulting from IκBα Y42 phosphorylation independent of IκB kinase (IKK). However, recent findings suggest that H_2O_2 activates NF-κB mainly through the classical IKK-dependent pathway (Gloire et al. 2006). ROS-induced NF-κB activation regulates inflammatory cytokines, chemokines, and adhesion molecule production, playing a role in the induction of inflammatory responses (Majkova et al. 2009). Binding sites for NF-κB and related transcription factors have been identified in the promoter regions of a variety of inflammatory genes (Muller et al. 1997; Kunsch and Medford 1999), such as interleukin 6 (IL-6), vascular cell adhesion molecule-1 (VCAM-1), and cyclooxygenase-2 (COX-2), all of which are upregulated by PCB exposure (Hennig et al. 2002; Kwon et al. 2002; Choi et al. 2003; Shimizu et al. 2007).

9.2.2 Non-dioxin-Like Pollutants

Non-coplanar PCBs, which contain ortho-substituted chlorine atoms, are often referred to as non-dioxin-like PCB congeners because they do not exhibit primary toxicity through the activation of AhR (World Health Organization 1998). Non-dioxin-like PCBs can be classified into additional subsets based on the varying mechanisms and pathways through which they exhibit toxicity, as serotonin biosynthesis inhibitors or as agonists/antagonists to estrogen receptors (ERs). Studies indicate that PCBs substituted with two or more chlorine atoms at the *ortho* positions can compete with natural ligand binding to ERs and androgen receptors (ARs) and thus interfere with sex hormone regulation (Bonefeld-Jorgensen et al. 2001). More specifically, lower chlorinated non-dioxin-like PCBs act as weak ER agonists while higher chlorinated non-dioxin-like PCBs act as weak ER antagonists, although health effects associated with these ER pathway effectors are not well known. PCBs may cause interference with sex steroid signaling, leading to overall reproductive health impairments and changes in newborn sex ratios (Brouwer et al. 1999; Meeker and Hauser 2010). PCB-induced thyroid hormone disruption also has been linked to hypothyroidism, which in turn affects neurodevelopmental outcomes such as psychomotor development, mental development, and memory (Brouwer et al. 1999; Trnovec et al. 2008).

In addition to non-dioxin-like PCBs, contaminants classified as endocrine-disrupting chemicals also include bisphenol A (BPA), polybrominated diphenyl

ethers (PBDEs), dichlorodiphenyltrichloroethane (DDT), and a variety of phthalates (Crinnion 2010). BPA, along with PCBs and phthalates, is a xenoestrogen that stimulates various cellular responses at very low concentrations (Rubin 2011). In some cases, BPA has been shown to exhibit equivalent binding potentials to estradiol, leading to BPA binding to classical and nonclassical membrane ERs as well as the G-protein-coupled receptor 30 (GPR30) membrane protein, which has high affinity for estradiol (Thomas and Dong 2006). BPA has been shown to interact with the ligand-binding domain of classical ERs and to recruit transcriptional coregulators in a distinct manner from estradiol, though, indicating that BPA is not solely an estrogen mimic (Gould et al. 1998; Routledge et al. 2000). In addition to acting as an estrogen, early-life BPA exposure alters estrogen sensitivity in a tissue-specific manner; perinatal BPA exposure alters the postnatal response to estradiol in mammary glands (Munoz-de-Toro et al. 2005; Wadia et al. 2007).

A hypothesized mechanism for the behavioral and neurodevelopmental toxic effects of PBDEs is its disruption of thyroid hormone regulation during critical developmental stages (Dishaw et al. 2014). PBDEs and their hydroxylated metabolites primarily target the thyroid system, depending upon their structural similarity to endogenous thyroid hormones, which is essential for cell migration and synaptogenesis in the brain and proper neurodevelopment procedures (Howdeshell 2002; Zoeller and Rovet 2004). Hydroxylated PBDEs bind to thyroid nuclear receptor and even to the estrogen receptors (Cao et al. 2010; Ibhazehiebo et al. 2011; Ren et al. 2013). In addition to the effects on neurodevelopment and thyroid hormone regulation, PBDE exposure has also been implicated in negative reproductive end points. For example, serum PBDE levels were associated with longer gestation and reduced fecundability (Harley et al. 2010).

DDT is an organochlorine pesticide linked with increased rates of abnormalities in male sexual development. The primary persistent metabolite of DDT, 2,2′-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE), exerts antiandrogenic activity by altering the expression of androgen-dependent genes (Kelce et al. 1995, 1997). While DDE is unlikely to bind to ERs, it inhibits androgen binding to the ARs as well as androgen-induced transcriptional activity (Kelce et al. 1995; Kelce and Wilson 1997).

Finally, phthalates, which are common plasticizers used in many consumer products, are gaining much attention for causing toxicity in humans. Phthalates are believed to produce endocrine-disrupting effects, as in utero exposure to these compounds induces developmental and reproductive toxicity (Lyche et al. 2009). Early studies reported several phthalates mimic estrogen by binding to ERα and inducing ERα-mediated gene expression. In addition, multiple phthalate diesters with alkyl chains exhibit not only estrogen receptor α-mediated estrogenic activity but also ERβ-mediated antiestrogenic activity (Harris et al. 1997; Zacharewski et al. 1998; Andersen et al. 1999; Takeuchi et al. 2005). Other phthalate diesters possess AR-mediated antiandrogenic activity through binding to ARs, although phthalate monoesters and esters with very short (diethyl) or very long (diheptyl) side chains seem to have no effect on AR activity (Takeuchi et al. 2005). Thus, phthalate esters simultaneously act as agonists and/or antagonists via one or more hormonal receptors, and interaction of phthalate esters with these receptors is contingent on the size of the ester side chains.

9.3 Epidemiological Links Between POPs Exposure and Noncommunicable Diseases

Although mechanisms by which POPs cause many of the conditions described below remain relatively unknown, significant relationships between these chemicals and various noncommunicable diseases have been established. Where possible, the influence of POPs on the development of specific health conditions is divided into that associated with dioxin-like and non-dioxin-like pollutants. It is important to distinguish between these two classes of compounds due to differences in their mechanisms of toxicity, but many studies measure POPs as a total sum of pollutants (e.g., studies that analyze the adverse health effects associated with PCB congener mixtures instead of individual congeners) and do not make this differentiation.

9.3.1 Diabetes Mellitus Type 2

Much research into the link between dioxin-like chemicals and type 2 diabetes has been in reference to US veteran exposure to the herbicide Agent Orange during the Vietnam War, which was contaminated with the highly toxic dioxin compound TCDD (Henriksen et al. 1997; Michalek et al. 1999; Longnecker and Michalek 2000). Many other dioxin-like POPs can also induce the development of diabetes. Examination of serum concentrations of POPs in a cross-sectional study of 2016 adults in the 1999–2002 National Health and Nutritional Examination Survey (NHANES) found that 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) and 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD) were also associated with instances of diabetes (Lee et al. 2006). Three additional cross-sectional studies of Japanese and Chinese participants also showed a positive relationship between dioxin-like POPs and metabolic syndrome, which is a risk factor for the development of diabetes (Wang et al. 2008; Uemura et al. 2009; Nakamoto et al. 2013). Other dioxin-like PCBs, specifically PCB 105 and PCB 118, also increased the incidence of diabetes in 725 seniors aged 70 in the Prospective Investigation of the Vasculature in Uppsala Senior study (Lee et al. 2011). There is also conflicting evidence on the correlation between dioxin-like PCBs and type 2 diabetes, as indicated by a cohort study of Great Lakes sport fish consumers wherein neither PCB 118 nor the total PCB mixture were associated with increased incidence of diabetes (Turyk et al. 2009). Data collected from the Coronary Artery Risk Development in Young Adults examined 22 separate PCB congeners and found that there was no association between incidence of type 2 diabetes and dioxin-like PCBs 105, 118, 156, 157, and 167 (Lee et al. 2010).

A cross-sectional survey of a heavily polluted area of eastern Slovakia showed that the presence of multiple POPs in serum, including PCBs, DDE, and DDT, increased the prevalence of diabetes in a pollutant dose-dependent manner (Ukropec et al. 2010). These findings contrast with a nested case-control study using participants in the Coronary Artery Risk Development in Young Adults (CARDIA) cohort.

This study found a reciprocal dose-dependent incidence of type 2 diabetes with participants exposed to lower doses of POPs exhibiting greater risk. Participants exposed to a higher concentration of PCBs demonstrated an inconsistent association with type 2 diabetes (Lee et al. 2010). The increased risk of developing type 2 diabetes may be sex dependent. A 24-year follow-up study of the Yucheng people of Taiwan, following an accidental exposure to rice bran oil laced with PCBs, yielded only in females an increase in incidences of type 2 diabetes (Wang et al. 2008). These findings were further substantiated by a study involving a cohort of exposed Michigan residents where women with higher levels of PCBs were 2–2.3 times more likely to develop type 2 diabetes (Vasiliu et al. 2006).

Non-dioxin-like POPs can also influence type 2 diabetes disease progression. In a cross-sectional study of obese and lean participants, total serum or adipose tissue levels of POPs, specifically PCB153, PCB138, and PCB180, were correlated with increased glucose levels, as tested by oral glucose tolerance tests, and hemoglobin A1c (HbA1c) levels. In this study, total POP levels had no effect on fasting insulin levels, indicating that there was no compensatory secretion of insulin, thus supporting the hypothesis that POPs exhibit a direct toxic effect on insulin-producing beta cells, resulting in the possible development of type 2 diabetes (Dirinck et al. 2014). Additional cohort studies involving PCBs 153 and 180 as well as DDE show a strong correlation between PCB concentration and incidence of type 2 diabetes, indicating that while different mechanisms of toxicity are at play, both non-dioxin-like and dioxin-like pollutants may result in increased development of type 2 diabetes (Lee et al. 2006, 2011; Turyk et al. 2009).

9.3.2 Cardiovascular Dysfunction and Disease

Residents of Anniston and Alabama have been the focus of much study into the health effects associated with POPs due to their proximity to a Monsanto Company factory that produced PCB mixtures called Aroclors for 40 years prior to their production ban in 1979. The risk of cardiovascular disease development has been extensively studied in this population, with findings indicating that elevated serum lipid concentrations are associated with elevated serum concentrations of overall PCBs, further implicating PCBs in the etiology of cardiovascular disease (Aminov et al. 2014). Additional studies involving Anniston residents found that total serum PCB concentrations were the strongest determinants of blood pressure, suggesting that PCBs may contribute to an increased risk of hypertension (Goncharov et al. 2010). While these studies implicate PCBs in cardiovascular disease, their differing modes of disease development further indicate that differing molecular geometries of PCB congeners work through differing molecular mechanisms to elicit toxic effects (Goncharov et al. 2010; Aminov et al. 2014).

Hospitalization rates for acute myocardial infarction in patients with diabetes were increased in populations living near sites contaminated with POPs (Sergeev and Carpenter 2010a). Previous studies that used a similar zip code proximity com-

parison study design showed analogous effects, in which patients in close proximity to sites of POP contamination were hospitalized due to stroke 10–15% more often over 7- to 12-year periods (Shcherbatykh et al. 2005; Sergeev and Carpenter 2010b). After adjustments for age, sex, race, and health insurance coverage, patients living near POP-contaminated sites also displayed significant increases in carotid artery plaque formation, a 15% elevation in coronary heart disease, and a 20% elevation in acute myocardial infarction (Sergeev and Carpenter 2005; Lind et al. 2012). The NHANES data set has also enabled multiple studies into the adverse effects of POPs on cardiovascular health. Increased rates of hypertension were seen in a cross-sectional study of 524 adults, aged 40 and above, where increased serum concentrations of PCDDs and PCDFs in female participants were correlated with newly diagnosed hypertension, while serum concentrations of PCBs (both dioxin-like and non-dioxin-like congeners) were correlated with increased rates of hypertension in men (Ha et al. 2009). A previous NHANES-related study that examined 889 adults (≥40 years old) also found that both dioxin-like and non-dioxin-like PCBs were positively associated with increased prevalence of self-reported cardiovascular disease in females, while PCDDs were implicated in increased rates of cardiovascular disease in both males and females (Ha et al. 2007).

Finally, another study showed that before adjustment for age, gender, race, smoking status, body mass index, exercise, total cholesterol, and family history of coronary heart disease, all PCBs examined were associated with instances of hypertension (Everett et al. 2007). It is also worth noting that several studies have indicated that race (Goncharov et al. 2010; Sergeev and Carpenter 2010a, b, c; Aminov et al. 2014), gender (Ha et al. 2007, 2009; Sergeev and Carpenter 2010a, b), and age (Sergeev and Carpenter 2010b, c) all play a role in risk assessment for development of cardiovascular dysfunction due to POP toxicity.

9.3.3 Obesity

Environmental influences on obesity and human health are of increasing interest because various environmental toxicants, including POPs and emerging contaminants, are now known to act as obesogens, i.e., chemicals that cause inappropriate receptor activation leading to increased adipocyte differentiation and development of obesity (Grun and Blumberg 2006). While decreased physical activity and poor nutrition are still the most important influencers of obesity development, environmental obesogens also modulate the human body's ability to manage its own weight control mechanisms (Baillie-Hamilton 2002).

In a study of 195 subjects, evaluation of PCB levels in serum and adipose showed preferential deposition of lipophilic POPs, such as the coplanar PCBs 153, 138, and 180, in adipose tissue of obese patients, which effectively decreased serum PCB levels as compared to lean individuals (Yu et al. 2011; Dirinck et al. 2014). Previous studies reported that weight gain was associated with higher PCB deposition in adipose tissue and thus lower concentrations of POPs in serum, while weight loss of 20 lb or more was associated with release of POPs back into the bloodstream,

yielding higher serum concentrations (De Roos et al. 2012). Adipose tissues collected from obese patients during bariatric surgery contained POPs in 96.3% of both human visceral and subcutaneous adipose tissue, with POP concentrations increasing with age and obesity duration (Pestana et al. 2014). While POPs are found in lipid-rich areas throughout the human body, current understanding does not indicate further location-specific POP deposition based on individual POP molecular composition and properties (Yu et al. 2011). However, the molecular composition of POPs plays a role in overall obesity risk, where pollutant body burden with higher concentrations of the more highly chlorinated PCB congeners leads to high development of adiposity (De Roos et al. 2012).

9.3.4 Cancer

Many of the POPs described previously are known carcinogens in animal models as well as in humans, based on findings from cell culture, in vivo studies, and most recently epidemiological studies. Similar to the diseases of chronic inflammation previously outlined, many of the epidemiological studies examining associations between POPs and cancer were conducted using populations exposed to abnormally high levels of POPs, where worker or population exposure is linked to their proximity to a plant releasing POPs as byproducts. In one such instance, the Seveso disaster (Italy, 1976), a chemical cloud containing several kilograms of TCDD was released into the surrounding area resulting in an increased rate of cancer development in the nearby population (Bertazzi et al. 2001; Pesatori et al. 2003; Consonni et al. 2008; Warner et al. 2011).

While there is currently limited evidence tying TCDD to cancer development in humans, other POPs, such as PCDF, PCDD, and PCBs, are implicated as potentially equal or greater risks for cancer development. A population in Japan exposed to PCBs and PCDF in 1968 exhibited increased rates in cancer-related mortality linked to proximity to the initial contamination site (Kashima et al. 2011, 2015). In addition, a 25-year study of Chinese workers exposed to PCDD and PCDF showed higher rates of cancer development and mortality in comparison to national averages, with a direct correlation drawn between concentration of exposure and rate of disease development (Wang et al. 2013a). Italian factory workers exposed to elevated levels of PCBs also exhibited increased mortality rates associated with biliary tract cancer in men, digestive tract cancer, and brain cancer, as well as increased risk for Hodgkin and non-Hodgkin lymphoma, further suggesting an association between PCBs and cancer (Pesatori et al. 2013). Limited epidemiological evidence also implicates PCB exposure in the development of breast cancer (Negri et al. 2003; Brody et al. 2007). In a case-control study of 112 postpartum serum samples from the Child Health and Development Study, exposure to PCBs elicited a congener-specific increase or decrease in the risk of breast cancer development. These associations suggest a more complicated underlying mechanism of toxicity (Cohn et al. 2012).

Studies examining non-Hodgkin lymphoma incidence in the Doubs region of France during 1980–2005 found that there was a steady increase from 1983 to 1992

across all age groups. The increase was associated with environmental exposure to PCBs beginning in the 1960s, which reached peak production during that time period (Viel et al. 2010; Kramer et al. 2012). Additional studies performed by the National Cancer Institute found that certain PCB congeners (PCBs 156, 180, and 194 measured in serum; PCBs 105, 138, 153, 170, and 180 measured in airborne samples) contributed to increased rates of non-Hodgkin lymphoma development (Colt et al. 2005; De Roos et al. 2005). Similar airborne sample collection techniques as above were used to examine the potential relationship between PCBs and an unexplained rise in the development of acute lymphocytic leukemia in children during 1975–2004. In a study of samples from 184 acute lymphocytic leukemia cases, ages 0–7, and control air samples from the primary residence, it was determined that the presence of PCBs in the residence conferred at least a twofold increase in the risk of developing acute lymphocytic leukemia, with the risk increasing as a function of the measured airborne concentrations of PCB 118, 138, and 153

Table 9.1 Epidemiological studies linking POPs and multiple cancer types

POPs of interest	Risk analysis technique[a]	Cancer types associated with POPs	Citation
TCDD	RR	All cancers (1.3), rectal (2.4), lung (1.3), Hodgkin lymphoma (4.9, after 10 years), non-Hodgkin lymphoma (2.8, after 15 years)	Bertazzi et al. (2001)
TCDD	SMR	All cancers (1.0), lung cancers (0.7), nonmalignant respiratory disease (0.8), leukemia (1.9), non-Hodgkin lymphoma (1.4)	Collins et al. (2009)
TCDD	SMR	All cancers (1.1), non-Hodgkin lymphoma (1.6), lung cancer (0.8)	McBride et al. (2009)
PCBs and PCDFs	SMR	All cancers (1.25, 15–19 years after exposure)	Kashima et al. (2011)
PCBs and PCDFs	SMR	All cancers (1.4, 30–34 years after exposure)	Kashima et al. (2015)
PCDDs and PCDFs	SMR	All cancers (1.70), lung cancer (2.13), and liver cancer (1.71)	Wang et al. (2013b)
PCBs	SMR	Biliary tract (3.91 in males), digestive cancer (2.54), brain cancer (2.13)	Pesatori et al. (2013)
PCBs	OR	Breast cancer: net PCB exposure (75th vs. 25th percentile = 2.8), PCB 203 (75th vs. 25th percentile = 6.3)	Cohn et al. (2012)
PCBs	OR	Non-Hodgkin lymphoma: PCB 156 (1.69), PCB 180 (1.08), and PCB 194 (1.45)	De Roos et al. (2005)
PCBs and DDE	OR	Non-Hodgkin lymphoma: any PCB (1.5), DDE (1.3, in males), top fertile of PCB 180 exposure (1.7)	Colt et al. (2005)
PCBs	OR	Acute lymphocytic leukemia: any PCB (1.97), total PCB (2.78, when lowest quartile compared to highest quartile of exposure)	Ward et al. (2009)

[a]Risk analysis performed using the following techniques: *RR* risk ratio, *SMR* standard mortality ratio, *OR* odds ratio

(Ward et al. 2009). There is a growing body of evidence that both dioxin-like and non-dioxin-like chemicals can initiate, promote, or advance the progression of multiple cancers (Table 9.1).

9.4 Antioxidant Therapy Reduces Inflammation and Associated Diseases

The use of antioxidants as clinical therapeutics has gained much attention, but their efficacies against multiple chronic diseases require future investigation. Since the discovery of antioxidant enzymes and ROS and their critical role in cellular homeostasis and human disease, researchers have been interested in counteracting their detrimental effects via dietary means. Therefore, antioxidants found in fruits and vegetables have been investigated for their protective properties in cell culture, animal models of disease, and human studies. Unfortunately, the convincing and exciting results from in vitro and in vivo studies have not translated to overwhelming success for human clinical patients. This section of the chapter will describe multiple clinical trials concerning the use of antioxidant therapies for modulating inflammation-related diseases, as well as emerging and future directions of this clinically relevant area of study.

9.4.1 Dietary Vitamins as Antioxidant Therapies

Vitamin E and vitamin A have been extensively studied during interventional studies as well as human prospective cohort studies (Wang et al. 2013b). Ultimately, more work needs to be done to determine if supplementation with single vitamins can prevent pro-inflammatory and pro-oxidative diseases such as diabetes, cancer, heart disease, and cancer.

9.4.1.1 Vitamin E

Vitamin E is a lipophilic compound found as tocopherol or tocotrienol forms, each with distinctive bioactive properties and bioavailabilities (Wang et al. 2013b). The Women's Health Study was a 10-year randomized trial that resulted in negligible protective effects of vitamin E on major cardiovascular events and cancer risks (Lee et al. 2005). This study did show a significant 24% decrease in cardiovascular-related deaths and a 26% decrease in major cardiovascular events in older women (at least 65 years of age). Other studies have concluded negligible protective effects of vitamin E supplementation on cardiovascular risks, but most studies do not stratify or report findings by age (Lee et al. 2005; Lonn et al. 2005). Vitamin E supplementation was ineffective in reducing the risk of type 2 diabetes but has been linked to decreased oxidative stress and improved glycemic conditions (Liu et al. 2006). Interestingly, it was determined

that women without genetic predisposition to type 2 diabetes (no family history) were modestly protected with vitamin E supplementation (Liu et al. 2006).

One reason that many human studies do not observe protective effects of vitamin E may be due to the importance and necessity of investigating subgroups of populations. In people genetically predisposed to higher levels of oxidative stress, individuals supplemented with vitamin E for 18 months showed reduced myocardial infarction, stroke, and cardiovascular deaths (Milman et al. 2008). This result may implicate vitamin E as protecting antioxidant against cardiovascular maladies in subpopulations that are prone to or at higher risk for increased oxidative stress. Finally, one study investigated vitamin E supplementation in diabetic patients but importantly stratified these individuals into type 1 and type 2 diabetes. Without this delineation, the researchers may have missed important results such as significantly decreased numbers of late complications like foot ulcers, retinopathy, and cardiovascular complications after 2 years (Baburao Jain and Anand Jain 2012). Although clinical studies of the efficacy of vitamin E suggest minimal protection against inflammatory diseases for the general public, more work needs to be done to examine if vitamin E may still be protective in some subpopulations.

9.4.1.2 Vitamin A

Vitamin A, a lipid-soluble antioxidant, is found in food sources either as retinol or as carotenes such as beta-carotene. Multiple studies, such as the Beta-Carotene and Retinol Efficacy Trial (CARET), have combined beta-carotene and retinol into a single intervention but with similar negligible or even detrimental effects (Omenn et al. 1996). The CARET study was halted prematurely when it was determined that individuals supplemented with the beta-carotene and retinol were at greater risk for lung cancer and cardiovascular disease. Studies on these subjects later indicated that the intervention might be linked to higher cholesterol and triglyceride concentrations (Cartmel et al. 2005). Multiple other cancer prevention studies have also resulted in minimal, if not detrimental, findings concerning vitamin A supplementation, but synthetic retinol-like compounds may hold promise (Fritz et al. 2011). Individuals highly exposed to lead were administered beta-carotene for 12 weeks and were examined for oxidative stress and antioxidant markers (Dobrakowski et al. 2014). Such supplementation decreased the oxidative stress marker and malondialdehyde and homocysteine levels (Dobrakowski et al. 2014).

9.5 Dietary Polyphenols and Flavonoids as Antioxidant Therapies

Polyphenols, such as resveratrol and catechins, are the most abundant plant dietary antioxidants available to humans (Manach et al. 2005). Epidemiological evidence points to inverse relationships between polyphenol consumption and chronic

inflammatory diseases such as cardiovascular disease. Observational studies have long identified that certain populations, such as those residing in or around France and other Mediterranean countries, that ingest relatively higher levels of bioactive polyphenols tend to be protected from obesity, type 2 diabetes, and cardiovascular maladies compared to other similarly Western developed countries (Schröder 2007; Martínez-González et al. 2015).

9.5.1 Resveratrol

Resveratrol has been shown to slow or prevent many age- and inflammation-related diseases such as diabetes and cardiovascular disease (Smoliga et al. 2011). Many in vitro and in vivo studies provided evidence for its antioxidant and anti-inflammatory effects. Most in vivo interventions investigate the acute effects of resveratrol supplementation (1 day to a few months) and the impact on inflammatory markers and risk factors for heart disease such as endothelial function, total antioxidant capacity, or oxidative stress levels (Manach et al. 2005). For example, one such 12-week intervention study showed that patients suffering from nonalcoholic fatty liver disease (NAFLD) displayed significant reduction in liver toxicity parameters, inflammatory cytokine levels, and extent of steatosis when supplemented with 500 mg resveratrol (Faghihzadeh et al. 2014). This protection was greater in patients who increased their physical activity (Faghihzadeh et al. 2014). Other short-term studies have looked at resveratrol's role in protecting against inflammation, obesity, diabetes, and cancer; however long-term studies are lacking (Smoliga et al. 2011).

Currently, clinical trials have investigated resveratrol's protective effects in patients who were at high risk for heart disease as well as those with established heart disease. These 1-year follow-up trials reported "improvement of the inflammatory, atherogenic, and fibrinolytic profile of these patients, and the absence of drug interactions or adverse effects," justifying future studies with larger sample sizes with even longer duration (Tome-Carneiro et al. 2012, 2013a, b). Without well-designed long-term intervention studies, it remains to be determined whether resveratrol is protective against oxidative stress-induced inflammatory diseases, as suggested previously by many researchers.

9.5.2 Catechins

The catechins and theaflavins found in readily available tea species have been linked to the prevention of cardiovascular diseases, diabetes, and cancer (Khan and Mukhtar 2013). Some of the strongest protective effects of tea consumption include decreases in obesity, body mass index, and related metabolic parameters. In a 60-day short-term intervention study in an elderly population, consumption of three cups of green tea a day resulted in weight loss and reduced body mass index and

waist circumference (Vieira Senger et al. 2012). In a comparable study in obese elderly Caucasian women, supplementation with 300 mg/day of green tea EGCG for 12 weeks did not enhance energy-restricted diet-induced adiposity reductions and did not improve weight loss (Mielgo-Ayuso et al. 2014). Although this result may seem contradictory, it may in fact exemplify the importance of consuming the wide variety of antioxidant polyphenols and flavonoids in tea and not simply one constituent part such as EGCG. Importantly, excess weight and obesity are related with type 2 diabetes, insulin resistance, and other diseases, so a weight loss effect due to tea consumption may be a critical component of protection (Hsu et al. 2011).

A large prospective study of Japanese individuals determined that drinking more than two cups and especially more than four cups of tea a day resulted in decreased risk for coronary heart disease and stroke (Kokubo et al. 2013). A separate 42-day intervention study reported that adding two cups of green tea a day to a controlled diet increased plasma total antioxidant activity, decreased plasma peroxides, and reduced LDL cholesterol (Erba et al. 2005). It was known that 30% of men that displayed high-grade prostate intraepithelial neoplasia would develop prostate cancer, but it was determined that in men supplemented with 600 mg/day of green tea catechins for 1 year, only 3% of men developed prostate tumors (Bettuzzi et al. 2006). Importantly, after the conclusion of the supplementation, a subset of these high risk men were again examined many months later, and the final difference in cancer prevalence between the two groups was highly significant, showing the long-lasting preventive effects of tea consumption against prostate cancer (Brausi et al. 2008). Therefore, long-term tea consumption may be a more effective preventative measure rather than short-term therapeutic use. However, different tea constituents could not decrease localization of prostate cancer or prostate-specific antigen (PSA) levels in certain patients (Jatoi et al. 2003; Khan and Mukhtar 2013).

9.6 Antioxidant Therapy Against the Toxicity of Environmental Pollutants

Utilizing dietary means to bolster human health is considered as a sensible way to protect against toxicity of environmental pollutants (Fig. 9.2). This section is adapted from reviews written by the authors (Petriello et al. 2014a, b) and supplemented with clinically relevant information where available. Diets rich in polyphenols have been correlated with decreased inflammation, metabolic syndrome, and atherosclerosis (Hennig et al. 2005, 2007). Food components, rich in antioxidants and anti-inflammatory agents, are often a large part of the French and Mediterranean diets (Nadtochiy and Redman 2011). As expected, these regions demonstrate a significant decrease in heart disease and related pathologies when compared to similar industrialized Western countries (Urpi-Sarda et al. 2012). The protective agents responsible for the decrease in oxidative stress, inflammation, and associated chronic diseases may include bioactive food components, such as resveratrol, quercetin, and catechins, working independently or synergistically (Moore et al. 2009;

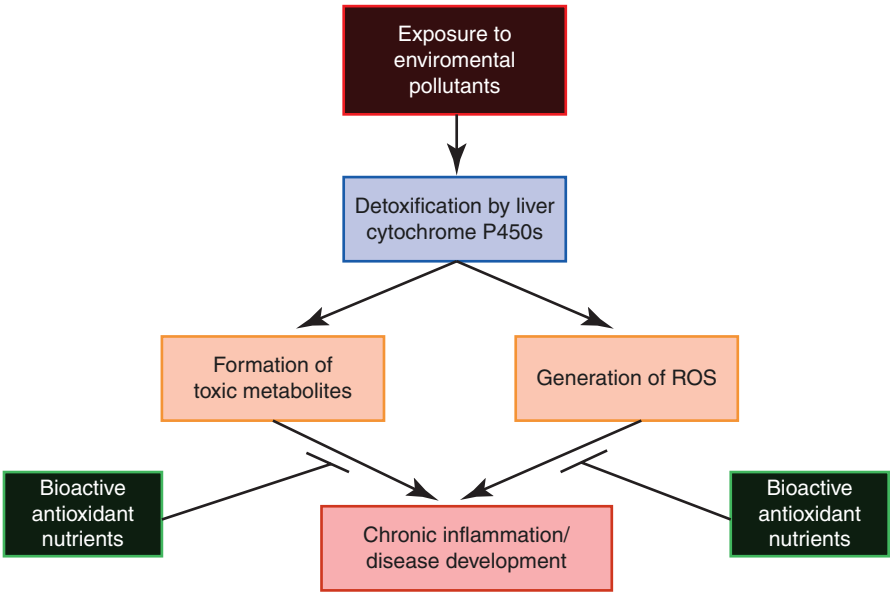

Fig. 9.2 Exposure to persistent organic pollutants can lead to chronic inflammation, but onset of disease may be modulated by bioactive antioxidants found in nutritious foods. Persistent environmental pollutants can enter the body via multiple means of exposure including ingestion of contaminated foods and through polluted air and water. To counteract negative health outcomes associated with daily exposure to a multitude of xenobiotics, the body has evolved many mechanisms of endogenous detoxification and protection. Many detoxification enzymes (i.e., cytochrome P450s) are found within the liver, but the process of toxicant elimination is not always efficient, as toxic metabolites and/or reactive oxygen species (ROS) can be created. To counteract the negative side effects of toxicant elimination, dietary bioactive antioxidants may directly scavenge ROS, induce multiple antioxidant enzymes, or increase the excretion rate or efficiency of detoxification, leading to decreased pollutant-induced inflammation and disease

Choi et al. 2010; Poudyal et al. 2011). Polyphenols may contribute to the bio-modulation of the increased oxidative stress and inflammation mediated by dioxin and non-dioxin-like pollutants. Phytochemicals found in foods protect against POP-related toxicity. Importantly, the ROS-scavenging polyphenols found in many fruits and vegetables decrease toxicity from dioxin and related dioxin-like PCBs (Terao 2009), increase PCB excretion rates (Morita et al. 1997), prevent AhR-induced inflammation (Han et al. 2012), limit body wasting (Ciftci and Ozdemir 2011), and decrease cellular dysfunction (Zheng et al. 2010). Resveratrol has been shown to limit the activation and subsequent pro-inflammatory signaling of AhR, the primary receptor of dioxin-like pollutants (Wu et al. 2001; Tutel'yan et al. 2003).

The inflammation induced by POPs such as PCBs occurs in multiple cell types other than endothelial cells, such as adipocytes. Mice administered with dioxin-like chemicals exhibited upregulation of pro-inflammatory adipokines, altered adipocyte differentiation, and dyslipidemia (Arsenescu et al. 2008). Evidence for the

impact of phytochemicals on POP-induced adipocyte toxicity is lacking, but a large body of evidence indicates beneficial effect of bioactive nutrients on adipose inflammation, suggesting these phytochemicals may be able to reduce POP-induced adipocyte toxicity. Curcumin, capsaicin, resveratrol, and EGCG have all been demonstrated to decrease adipose inflammation and adipocyte oxidation (Leiherer et al. 2013), with resveratrol having the added benefit of modulating pro-inflammatory mediators, such as Toll-like receptor 4 (TLR4), which attenuates adipogenesis and adipose inflammation (Kim et al. 2011). Physiological inflammation is a diverse phenomenon encompassing multiple tissue types, but, importantly, many cell types prone to inflammation have devised endogenous protective pathways to decrease oxidative stress while balancing inflammation. Using bioactive food components that activate these pathways prior to toxicological insult may allow the body to more efficiently detoxify or eliminate environmental pollutants (Petriello et al. 2014b).

Data from animal models implicating flavonoids and polyphenols as having a protective role by possibly inducing antioxidant enzyme pathways as well as increasing fecal excretion of POPs have helped fill the information gap created by the lack of human studies (Morita et al. 1997; Newsome et al. 2014). These studies suggest important roles of plant-derived polyphenols. The first is to protect against POP-mediated oxidative stress, inflammation, and toxicity using host mechanisms, while the second is binding to POPs, therefore assisting in decreasing body burden. In fact, it has been proposed that green tea can inhibit the intestinal absorption of lipids and highly lipophilic organic compounds and accelerate excretion of PCBs (Khoo and Freeman 2010; Kim et al. 2011).

9.7 Protective Antioxidant Cellular Signaling Pathways

Although the mechanisms underlying nutritional modulation of toxicity induced by environmental toxicants have been attributed to a variety of signaling pathways, the antioxidant master controller nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling pathway may be the most pertinent when discussing the protection by antioxidants, mainly polyphenols (Fig. 9.3). Nrf2 is a transcription factor upregulating protective genes in response to oxidative stress, xenobiotics, and bioactive food molecules (Itoh et al. 2010; Kim et al. 2010; Singh et al. 2010). Many antioxidants, including resveratrol, sulforaphane, and EGCG, have been shown to activate Nrf2 (Nair et al. 2010; Kang et al. 2012; Miao et al. 2012). There are multiple mechanisms of Nrf2 activation, including phosphorylation of Nrf2 by PKC delta and loss of connection between Nrf2 and inhibitory Kelch-like ECH-associated protein 1 (Keap1) (Niture et al. 2009). Upon activation, Nrf2 is able to evade ubiquitination, translocate into the nucleus, and bind *cis*-acting antioxidant response elements in target genes (Baird and Dinkova-Kostova 2011). The activation of Nrf2 leads to a decrease in inflammation, which is often associated with POP-induced toxicity

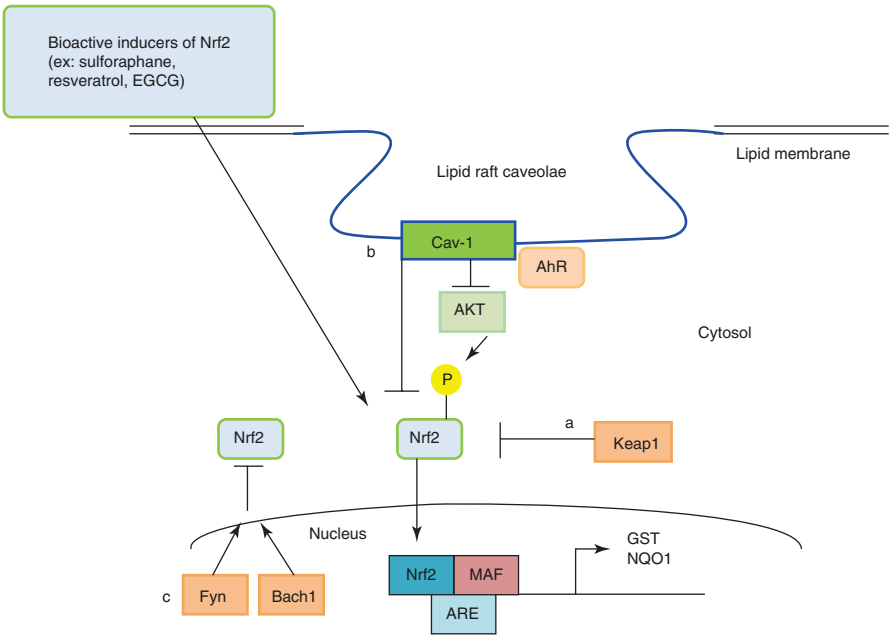

Fig. 9.3 The induction of Nrf2 is controlled by multiple inhibitory proteins and a complex regulatory mechanism. (*a*) Keap1 is the major inhibitory protein of Nrf2 and facilitates Nrf2's ubiquitination and destruction. (*b*) Caveolin-1, the major structural protein of lipid raft caveolae, binds and inhibits Nrf2. Also, caveolae concentrate other proteins involved in Nrf2 activation such as Akt which is known to phosphorylate and activate Nrf2. Cav-1 is also a binding partner of the aryl hydrocarbon receptor which is known to bind *cis*-acting sequences in the Nrf2 promoter. (*c*) Upon Nrf2 activation and subsequent translocation to the nucleus, additional regulatory mechanisms exist to remove Nrf2. Direct phosphorylation from Fyn kinase or Bach1 decreases the Nrf2 nuclear pool. If Nrf2 evades cellular destruction and remains in the nucleus, the transcription factor can bind co-activators, bind *cis*-acting antioxidant response elements on DNA, and upregulate a battery of antioxidant related proteins such as GST and NQO1

(Kim et al. 2010) therefore leading to vascular protection. Diets rich in polyphenols capable of activating Nrf2 play a role in prevention of inflammation. In addition, Nrf2 interacts with AhR, which is an important signaling factor in dioxin-like POP toxicity (Hayes et al. 2009). Although it has been known that dioxin and dioxin-like compounds activate both AhR- and Nrf2-related genes, it was shown that Nrf2 is necessary for induction of AhR target genes such as CYP1A1 (Yeager et al. 2009). Importantly, this cross-talk can be explained at the genetic level because the promoter region for Nrf2 contains AhR-binding regions and the gene promoter for AhR contains multiple Nrf2-binding elements (Hayes et al. 2009). These findings highlight the importance of a diet rich in polyphenols and flavonoids. The addition of these antioxidants may protect against POP-induced toxicity by possible synergistic activation of protective pathways or by simultaneously downregulating pro-inflammatory pathways while also scavenging ROS.

9.8 Intervention Studies and Antioxidant Roles of in the Modulation of POP-Induced Diseases

Well-designed human intervention studies that investigated protective roles of antioxidants against environmental pollutant-induced oxidative stress and diseases are lacking. In fact, the only such intervention studies have been conducted in workers with high occupational exposures to pollutants. For example, workers exposed to the POP, benzene, develop cancers and other maladies related to the reproductive, nervous, and immune systems. To counteract such toxicity, investigators supplemented these workers with six cups of green tea daily and monitored them for a 6-month period. In these supplemented workers, levels of benzene and its reactive metabolites, trans,trans-muconic acid, and phenol, were decreased compared to control un-supplemented workers (Emara and El-Bahrawy 2008). Comparable study that investigated the modulation of metal toxicity using nutritional interventions in male workers highly exposed to lead has shown that supplementation with beta-carotene for 12 weeks decreased lead-induced oxidative stress and increased activity of SOD (Kasperczyk et al. 2014).

Exposure to environmental pollutants was identified as major cause of health risk throughout the world (Briggs 2003). The global population and human activities have increased body burdens of POPs, such as PCBs, dioxins, and pesticides. Although a few preventative or therapeutic treatments are available, proof-of-principle studies have shown other approaches such as the use of a nonabsorbable fat substitute, olestra, which can decrease the body burdens of a wide variety of toxicants in actual human populations (Jandacek et al. 2010, 2014). Although the associations between environmental pollution, oxidative stress, and health outcome are poorly characterized, intervention strategies on antioxidant-based whole diets are needed given the possibility of decreasing body burdens of environmental pollutants but to the myriad of possible positive effects described above. Recent literature on prevention of diseases suggests that nutritional intervention strategies should be developed throughout early life, especially during the prenatal development period (Kaur et al. 2013; Al-Gubory 2014; Paparo et al. 2014; Taylor et al. 2014). This may most effectively reduce changes in pathologies triggered by in utero various human-made environmental pollutants.

9.9 Conclusions

POPs induce or exacerbate multiple disease phenotypes related to chronic oxidative stress and inflammation. Both dioxin-like and non-dioxin-like chemicals have been linked epidemiologically to obesity, heart disease, diabetes, and cancers. Although occupational exposures may elicit strong acute toxicities, the entire global population is exposed to multiple POPs. The extent of exposure is large enough to produce toxic effects throughout a lifetime.

Although environmental science- and engineering-based remediation technologies exist, these strategies can be time-consuming, detrimental to the environment, and cost prohibitive. Therefore it is critical to identify and develop biological strategies, such as dietary approaches to protect against chronic disease risks associated with environmental pollutants. Designing antioxidant-based intervention strategies may be efficacious means of modulating the toxicities of POPs and other environmental pollutants such as heavy metals. Antioxidant-rich foods may contribute to cellular protection against pollutant-induced inflammation by upregulating endogenous antioxidant cellular defenses, increasing excretion rates of POPs, and/or downregulating pro-inflammatory signaling cascades. Currently, limited information is available on populations with sub-toxic body burden levels of POPs, but as more information becomes available through long-term clinical studies, dietary interventions with antioxidant therapies may prove to be effective in preventing POP-induced human disease.

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Chapter 10
Antioxidants in Physical Exercise and Sports Performance

Li Zuo, Tingyang Zhou, and Chia-Chen Chuang

Abstract Skeletal muscle contraction generates reactive oxygen species (ROS), which are signaling molecules involved in exercise and force generation. Although ROS levels are maintained at physiological levels by endogenous antioxidants, exercise can alter the oxidant-antioxidant balance in contracting muscles. Regular exercise strengthens the antioxidant defense system via ROS-mediated adaptive responses, while strenuous exercise induces ROS accumulation and oxidative stress. Excess ROS level damages intracellular components and impairs muscle function, potentially limiting physical performance. The manipulation of antioxidant status can restore redox homeostasis and reduce exercise-induced oxidative damages. However, the effectiveness of antioxidant supplementation is unclear due to the complicated and multifaceted roles of ROS in both exercise-induced oxidative injuries and adaptation. The intensity, duration, and types of exercise are also likely to contribute to the effect of ROS in exercise. This chapter provides an updated discus-

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sion on ROS and antioxidants in aerobic and anaerobic exercises as well as their multifaceted effects on oxidative balance and physical performance.

Keywords Aerobic exercise • Anaerobic exercise • Exercise-induced adaptation • Oxidative stress • Antioxidant supplementation

10.1 Introduction

The findings that exercise enhances oxidative damage in animals and humans were first reported around the late 1970s (Brady et al. 1979; Dillard et al. 1978). Since then, several studies reported oxidant production in contracting skeletal muscle and various degrees of oxidative stress during exercise (He et al. 2016; Reid 2016). Ongoing research reveals that contraction-induced reactive oxygen species (ROS) participates in the regulation of muscle function and exercise-associated adaptive responses. Under normal conditions, the reactive properties of oxidants (e.g., short half-lives) and the presence of endogenous antioxidant defenses maintain ROS at physiological levels, which are essential in modulating muscle force production (He et al. 2016; Powers et al. 2011). However, oxidative damage-related contractile dysfunction and muscle fatigue can occur when ROS levels exceed the capacity of the antioxidant system during irregular or strenuous and sustained exercise (He et al. 2016; Reid 2016). For example, hydrogen peroxide (H_2O_2), a relatively long-lived ROS, is produced continually within the skeletal muscle. Its bioavailability influences muscle microvascular oxygenation and modulates myogenic tone during contractions (Hirai et al. 2011). Exercise elevates H_2O_2 production likely due to enhanced vascular shear stress and oxidative metabolism (Lauer et al. 2005; Wang et al. 2015). Such increase in H_2O_2 can lead to the reduction of myofibrillar Ca^{2+} sensitivity, thereby depressing force generation and impairing muscle contractility at cellular levels (Hirai et al. 2011; Moopanar and Allen 2006).

Several antioxidant interventions are proposed to alleviate oxidative stress and improve muscle performance but with conflicting results. Alternatively, regular exercise training increases the expression of key antioxidants in the skeletal muscle in response to elevations of ROS levels (Leonardo-Mendonça et al. 2014). ROS are involved in exercise-associated skeletal muscle adaptation by inducing various redox-sensitive signaling pathways such as the nuclear factor erythroid 2-related factor (Nrf2)-antioxidant response element pathways. The activation of these pathways enhances antioxidant expression and mitochondrial biogenesis, thus accommodating the oxidative environment of contracting skeletal muscles (He et al. 2016). Antioxidant supplementation interferes with exercise-induced adaptation and muscle function restoration (He et al. 2016; Reid 2016). Despite advanced understanding of redox biology in the skeletal muscle and exercise, significant gaps still exist, and research continues to resolve the complex effects of oxidative stress on physical

activity and oxidant-mediated muscle force control. In this chapter, we provide an updated discussion of ROS and antioxidant in aerobic and anaerobic exercises, as well as their multifaceted effects on oxidative balance and physical performance.

10.2 Aerobic Versus Anaerobic Exercise

Exercise can be classified into different types based on their energy generation pathways (aerobic and anaerobic), frequency (acute and chronic), or muscle contraction (isometric, eccentric, and concentric). The major difference between anaerobic and aerobic exercise is their distinct pathways for energy generation (Gomes et al. 2012). During anaerobic activity, adenosine triphosphate (ATP) is generated at a much faster rate compared to aerobic exercise, and there is an accumulation of lactic acid in muscles. This anaerobic process of ATP replenishment can be achieved via the transfer of phosphate from creatine phosphate to adenosine diphosphate (ADP) or via glycogen degradation to generate lactate (Baker et al. 2010). Every exercise activity begins with an anaerobic process, but prolonged periods of exercise can only be maintained aerobically. It takes an average of 2–3 min to invoke aerobic and anaerobic metabolism to yield equal energy levels during steady-paced running. During short-term anaerobic exercise or at the start of any physical work, ATP is primarily synthesized from the oxygen-independent glycolysis pathway, generating nicotinamide adenine dinucleotide (NAD^+) and lactate as by-products. As tissue oxygen (O_2) levels rise, the remaining pyruvate molecules enter mitochondria and participate in the Krebs cycle to generate more ATP molecules through oxidative phosphorylation. When aerobic respiration eventually takes over as the major mechanism of ATP synthesis during prolonged exercise, lactate production is then lowered (Baker et al. 2010).

Skeletal muscle fibers have distinct metabolic and contractile properties (Zierath and Hawley 2004). Type I (or slow) fibers, for example, have a large number of mitochondria and primarily use aerobic oxidative phosphorylation to generate ATP. Therefore, type I fibers are characterized by slow contractile properties, high fatigue resistance, and large aerobic capacities and are mostly recruited during endurance and aerobic exercise. On the other hand, type II (or fast) fibers are typically used for high-intensity work. There are three categories of type II fibers including type IIA, type IIB, and type IIX (Schiaffino and Reggiani 2011). Type IIB fibers have very low mitochondrial content, relying on anaerobic respiration as their main energy source, while type IIA fibers possess both high aerobic and anaerobic capacities (Wang et al. 2004). Therefore, type IIA fibers have fast-contracting and fatigue-resistant properties. By contrast, fiber IIB is fast but fatigue sensitive (Tellis et al. 2004). Type IIX fibers have similar twitch properties to those of type IIA and IIB muscles. However, their fatigue resistance is higher than type IIB but lower than type IIA fibers (Schiaffino and Reggiani 2011). In human muscles, the mitochondrial content in type I, type IIX, and type IIA fibers was estimated to be 6%, 4.5%,

Table 10.1 Muscle fiber types and properties

	Slow twitch	Fast twitch		
	Type I	Type IIA	Type IIB	Type IIX
Main energy source	Oxidative phosphorylation	Oxidative phosphorylation and anaerobic respiration	Anaerobic respiration	Anaerobic respiration
Mitochondrial content	High	High	Low	Moderate
Glycolytic enzymes	Low	High	High	High
Oxidative enzymes	High	High	Low	Moderate
Contractility	Slow	Moderately fast	Very fast	Fast
Fatigue resistance	Very high	High	Low	Moderate
Activity type	Endurance and aerobic exercise	High-intensity exercise	High-intensity exercise	High-intensity exercise

and 2.3%, respectively. Both type IIA and type IIB fibers contain abundant glycolytic enzymes. However, type IIA fibers have a much higher oxidative enzyme content than type IIB fibers (Schiaffino and Reggiani 2011) (Table 10.1).

10.3 Reactive Oxygen Species in Exercising Skeletal Muscle

Major ROS found in the skeletal muscles such as superoxide ($O_2^{\bullet -}$), H_2O_2, hydroxyl radicals ($^{\bullet}OH$), and peroxynitrite increase during muscular activity, although the cellular sites of their production are unclear (Powers et al. 2011, 2016). Measurements of oxidative stress in muscles have yielded inconsistent results, in part due to methodological shortcomings. The reactive nature of ROS likely complicates the direct and accurate quantification, as their alterations in contracting muscle fibers may be subtle. The limited specificity of ROS indicators and indirect measurements of oxidative stress are also subject to experimental artifacts (He et al. 2016). In addition, it is worth noting that variations in the sources and degrees of ROS production exist with different experimental types, intensities, and duration. Thus, studies on oxidative stress and exercise should be performed cautiously, and the interpretation of results may not be generalizable.

ROS are naturally produced in biological systems where they act as signaling molecules in various cellular pathways such as the preconditioning mechanism, gene expression, and immune response, whereas overproduction of ROS causes oxidative damage to intracellular components (Zuo et al. 2013, 2015a, b). For example, ROS can interrupt potassium transporters on the membranes, perturb cell signal transduction, and ultimately lead to compromised muscle force generation (Finaud et al. 2006). Mitochondria are widely regarded as the sources of intracellular ROS

in the skeletal muscle (Powers et al. 2016; Zuo et al. 2015a, b). The enzymes NADPH oxidase (NOX) and endothelial xanthine oxidase (XO) are also key ROS generators in contracting skeletal muscle. Among these, NOX contributes larger amounts of $O_2^{\bullet-}$ than mitochondria do during muscle contractions. It has been suggested that mitochondria are not the primary sites for cellular ROS production during muscle contraction since higher levels of ROS are generated during the resting state 4 of mitochondrial respiration compared to that in the active state 3 (He et al. 2016). Yet, there is a two- or threefold increase in mitochondrial ROS production (e.g., H_2O_2 release) in type II fibers (Powers et al. 2011). Two major NOX isoforms present in the skeletal muscle are NOX2 and NOX4. They are located in the T tubules, sarcolemma, and sarcoplasmic reticulum of muscle fibers and play an important physiological role in modulating muscle excitation-contraction coupling (Loureiro et al. 2016). NOX-induced ROS enhances muscle contractions by activating ryanodine receptors (RyRs) and subsequently initiating Ca^{2+} release (He et al. 2016). ROS also regulates the exercise pressure reflex (EPR), a regulatory mechanism that controls blood pressure during exercise. An attenuated EPR response occurs in skeletal muscles treated with NOX inhibitors (Wang et al. 2009). ROS formation is increased by overactive NOX in response to elevated levels of cytoplasmic Ca^{2+} (Powers et al. 2010; Whitehead et al. 2006; Yan et al. 2006).

The regulation and activity of extracellular ROS are somewhat different from that of intracellular ROS. It has been suggested that extracellular ROS in the skeletal muscle is released from intracellular sources such as mitochondria. However, inhibition of mitochondrial complexes I–III, NOXs, and cell membrane anion channels does not reduce extracellular ROS levels, suggesting other unknown sources of ROS in the contracting skeletal muscle (Zuo et al. 2015a, b). Prolonged and repetitive muscle contractions induce a substantial accumulation of ROS, thereby contributing to muscle fatigue and limiting physical performance (Reid 2016). Muscles can also be subjected to heat stress-induced ROS generation as body temperature increases during intensive exercise. For instance, the continually contracting diaphragm can be overworked during exercise due to increased respiratory demand. As a result, mitochondrial uncoupling leads to the formation of $O_2^{\bullet-}$ when the leaked electrons react with O_2 (Zuo et al. 2015a, b). Studies of the skeletal muscle undergoing heat stress show increase in both intracellular and extracellular $O_2^{\bullet-}$ release (Close et al. 2005; Zuo et al. 2000, 2014) (Fig. 10.1). In addition, lipoxygenase is another important source of $O_2^{\bullet-}$ in the skeletal muscle during heat stress (Zuo et al. 2004).

Intense bouts of exercise can shunt blood from tissues to skeletal muscles due to vasodilation responses to muscle contraction (exercise hyperemia), resulting in a condition resembling tissue ischemia (I), which is followed by tissue reoxygenation (R) when exercise ceases (Bloomer and Goldfarb 2004; Gomes et al. 2012; Joyner and Casey 2015). Ischemia can markedly elevate circulating XO, which catalyzes the reaction from hypoxanthine to xanthine with the generation of $O_2^{\bullet-}$ and H_2O_2 (Gomes et al. 2012). Evidently, anaerobic exercise-induced tissue damage is similar to other I/R-related injuries (Bloomer and Goldfarb 2004). Muscles in people undergoing maximum sprinting are likely to experience total deoxygenation (hypoxia) and hypoxia-induced muscle contracture (Nioka et al. 1998) (Fig. 10.1).

Clanton et al. observed contracture in the muscle exposed to H_2O_2 (>50 μM), which was prevented by treatment with antioxidants (Clanton 2007). Inflammation also contributes to ROS production during anaerobic exercise, particularly in aging populations. Although inflammatory responses play critical roles in removing infections and damaged proteins, ROS released from immune cells results in a series of secondary damage to tissues (Bloomer and Goldfarb 2004; Gomes et al. 2012). Paradoxically, regular and moderate physical activities are correlated with lower levels of inflammatory markers such as interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), and C-reactive protein. This highlights the importance of exercise intensity and frequency in regulating inflammation (Woods et al. 2012) (Fig. 10.1).

Despite the detrimental effects of ROS, modest or low levels of ROS are necessary for normal muscle force generation by eliciting intracellular Ca^{2+} release via the activation of RyR and the inhibition of sarcoplasmic Ca^{2+} ATPase activity (Powers and Jackson 2008; Powers et al. 2011). Espinosa et al. proposed that depolarization or electrical stimulation can initiate ROS generation in skeletal muscle cells and in turn lead to slow Ca^{2+} transients. ROS-dependent slow Ca^{2+} transients, which differs from fast Ca^{2+} transients, activate extracellular signal-regulated kinase (ERK) pathways without initiating muscle contraction (Espinosa et al. 2006). Other

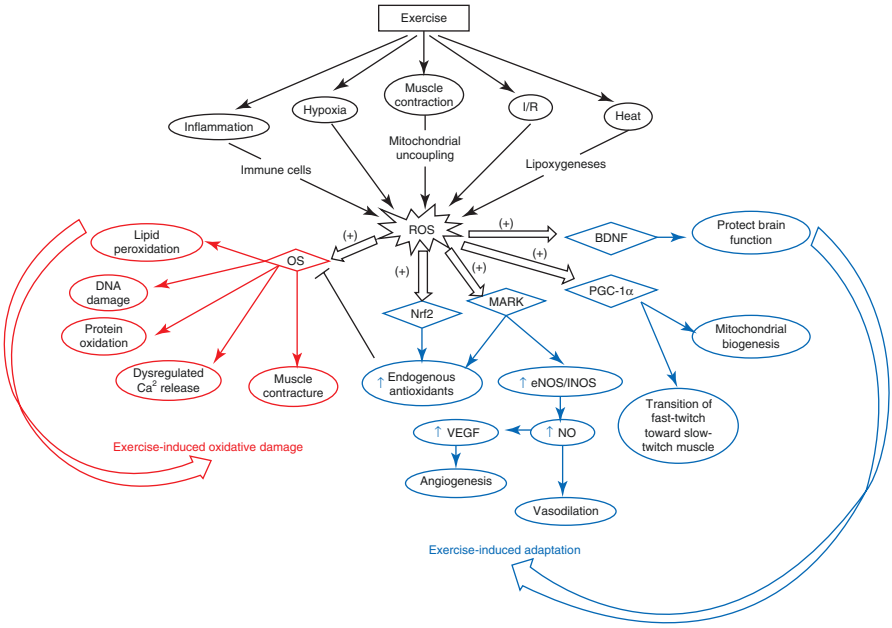

Fig. 10.1 Schematic illustrating the roles of ROS in exercise-induced oxidative damage and adaptation. *BDNF* brain-derived neurotrophic factor, *eNOS* endothelial nitric oxide synthase, *iNOS* inducible nitric oxide synthase, *I/R* ischemia reperfusion, *MAPK* mitogen-activated protein kinases, *NO* nitric oxide, *Nrf2* nuclear factor erythroid 2-related factor, *OS* oxidative stress, *PGC-1α* proliferator-activated receptor-γ coactivator-1α, *ROS* reactive oxygen species, *VEGF* vascular endothelial growth factor

studies also indicated that ROS-induced Ca^{2+} transients play a role in triggering mitogen-activated protein kinase (MAPK) pathways, including ERK1/2, p38 MAPK, and Jun amino-terminal kinases (JNK) in the skeletal muscle. The triggering of MAPK cascades is implicated in exercise-related adaptation which will be discussed later (Gómez-Cabrera et al. 2005; Long et al. 2004).

10.4 Oxidative Damage in Aerobic Versus Anaerobic Exercises

Both aerobic and anaerobic exercises increase ROS generation. However, exercise-induced oxidative stress is not necessarily restricted to muscular tissues (Gomes et al. 2012; Shi et al. 2007). Aerobic exercise of high intensities (>70% maximal O_2 uptake (VO_2 max)) and long durations (>30 min) leads to cellular oxidative stress (Belviranlı and Gökbel 2006), as shown by the elevated tissue levels of oxidized biomolecules following strenuous or prolonged aerobic exercises (Bloomer et al. 2010; He et al. 2016). For instance, a 50-km ultramarathon dramatically increases plasma levels of F_2-isoprostanes (F_2-IsoPs; a lipid peroxidation marker) (Mastaloudis et al. 2004). Significant increases in plasma levels of protein carbonyls, lipid hydroperoxides (LOOH), and the oxygen radical absorbance capacity occur in humans after exhaustive aerobic treadmill exercise (Alessio et al. 2000). Although aerobic exercise causes a drastic increase in O_2 consumption, ROS generation can be reduced during aerobic activities (Barja 2007), and the mitochondria only account for a small fraction of total ROS formation (He et al. 2016). Instead, XO plays a more important role in ROS generation during exhaustive aerobic exercises (Gómez-Cabrera et al. 2008b) as shown in a study where the administration of allopurinol (a XO inhibitor) reduced plasma markers of muscle damage following exhaustive exercise (Gómez-Cabrera et al. 2003).

Upregulated oxidative markers occur in various modes of anaerobic exercise including isometric, eccentric, and sprint exercise (Bloomer and Goldfarb 2004). Alessio et al. reported an immediate elevation in LOOH following isometric handgrip exercises; however, only a slight increase (~12%) in protein carbonyls was observed, suggesting that isometric handgrip exercise at this level of intensity caused severe lipid peroxidation but that was insufficient to influence protein oxidation (Alessio et al. 2000). Another study which utilized a 3-min dynamic handgrip exercise as the anaerobic mode reported increased plasma levels of thiobarbituric acid reactive substances (TBARS, an indirect marker of lipid peroxidation) and decreased levels of glutathione (GSH) (Steinberg et al. 2002).

The effects of oxidative stress during eccentric exercise (muscle lengthening) have also been examined. For instance, eccentric exercise causes oxidative DNA damage, as reflected by a significant increase in 8-hydroxy-2′-deoxyguanosine (8-OHdG) following 200 eccentric actions on the rectus femoris (Radák et al. 1999). Lee et al. observed marked increases in protein carbonyls following 60 eccentric muscle actions using elbow flexors (Lee et al. 2002). In contrast, Stagos et al.

reported that eccentric muscle actions with knee extensors did not induce significant changes in oxidative stress markers, such as TBARS and protein carbonyls, in either erythrocyte or in plasma (Stagos et al. 2015). The mixed results from eccentric exercise studies could be attributed to the differences in the biomarkers measured, the assay techniques, and the intensities of muscle action. Eccentric exercise-induced muscle injury and the resulting ROS overproduction are likely associated with inflammation and the interruption of Ca^{2+} homeostasis (Bloomer and Goldfarb 2004). Moreover, anaerobic sprints are associated with oxidation of lipids, protein, and DNA in most studies (Fig. 10.1).

Groussard et al. observed increased lipid radical levels in the serum of individuals after performing a single-sprint exercise (Wingate anaerobic test), as detected by electron spin resonance spectroscopy (Groussard et al. 2003). Another study showed that repeated-sprint exercise elevated plasma malondialdehyde (MDA; a lipid peroxidation marker) and lowered red blood cell GSH concentrations (Deminice et al. 2013). These studies suggested that anaerobic exercise leads to ROS overproduction and subsequently causes oxidation of lipids, proteins, and DNA within a short period of time. However, as opposed to these results, studies by Ihara et al. showed that anaerobic exercise does not cause any damage to the skeletal muscle. In their study, untrained healthy men performed exercise on a treadmill with increasing workload until they reached 100% VO_2 max. Within 3 min after the exercise, their blood was collected for analysis of muscle oxidative damage (Ihara et al. 2001). These results, which had been corrected for plasma volume changes, showed no significant differences in the levels of creatine kinase-M isoform (CK-M) and myoglobin before and after exercise. Since increased CK-M and myoglobin are common indicators of muscle damage, their results suggest that anaerobic exercise did not impair skeletal muscles (Ihara et al. 2001). The mixed results regarding the damaging effects of anaerobic exercise on the skeletal muscle are likely due to differences in exercise intensity.

10.5 Exercise-Induced Reactive Oxygen Species Production and Skeletal Muscle Adaptions

Regulation of redox balance in contracting skeletal muscle is essential for avoiding oxidative stress and subsequent tissue damage. Under physiological conditions, intracellular nonenzymatic and enzymatic antioxidants function to protect the skeletal muscle against exercise-induced oxidative damage by restoring redox homeostasis (He et al. 2016). Endogenous antioxidants include superoxide dismutase 1 (SOD1 or Cu/Zn-SOD, a major cytoplasmic scavenger of $O_2^{\bullet-}$), SOD2 (Mn-SOD, the principal scavenger of mitochondrial $O_2^{\bullet-}$), catalase (CAT), glutathione peroxidase (GPX), GSH, as well as other dietary antioxidants such as β-carotene and vitamins A, C, and E (Kang et al. 2009). SOD mainly catalyzes the conversion of $O_2^{\bullet-}$ to H_2O_2, which is less reactive but can be toxic at high levels. CAT and GPX are responsible for converting H_2O_2 to nontoxic products such as O_2 and H_2O. Specifically, GPX employs GSH as a substrate to reduce H_2O_2 to H_2O with the formation of glutathione disulfide (GSSG) (Zuo et al. 2015a, b). Ancillary enzymatic antioxidants such as

thioredoxin (TRX), peroxiredoxin (GRX), and glutaredoxin (PRX) systems also play essential roles in maintaining redox balance. TRX antioxidant system, which is composed of TRX and thioredoxin reductase, prevents protein oxidation. Thioredoxin reductase further reduces hydroperoxides and recycles vitamin C. With some overlapping functions, GRX primarily works to protect thiol oxidation during oxidative stress, while PRX reduces hydroperoxides and peroxynitrates (Powers et al. 2011).

Vitamin E (tocopherol) is a lipid-soluble antioxidant that exerts protective effects mainly on membranes by reacting with ROS directly. Vitamin C (ascorbic acid) is a water-soluble antioxidant that is present in both intra- and extracellular compartments. It neutralizes free radicals, such as $O_2^{\bullet -}$, $^{\bullet}OH$, and fatty acid radicals ($LOO^{\bullet}$) extracellularly while facilitating the activities of vitamin E and GSH intracellularly (Gomes et al. 2012). The relative amount of these antioxidants varies across muscle fiber types. For example, oxidative type I fibers express higher SOD and GPX activities as well as greater GSH levels than type II fibers (Powers et al. 2011; Steinbacher and Eckl 2015). It is likely that the extent of the adaptive upregulation of endogenous antioxidants following exercise is also fiber type-specific (Powers et al. 2011; Steinbacher and Eckl 2015).

Exercise training can alter the antioxidant capacity of the skeletal muscle. Such changes are dependent on the duration and intensity of the specific exercise. The benefits of regular exercise include the stimulation of downstream adaptive pathways and the promotion of oxidative stress resistance. Irregular or exhaustive exercise, on the other hand, results in extensive ROS production that overwhelms muscle antioxidant defenses. The resulting oxidative stress impairs muscle contractility and function (He et al. 2016). Intriguingly, exercise-induced ROS generation is recognized as an important signaling process in the skeletal muscle during exercise, and the supplementation of antioxidant abolishes such effects (Reid 2016). Training-induced increases in peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and mitochondrial biogenesis are diminished in both rat and human skeletal muscles following daily administration of vitamin C (Gómez-Cabrera et al. 2008a, b; Ristow et al. 2009). Similarly, allopurinol (a XO inhibitor) treatment attenuates exercise-associated PGC-1α upregulation as well as elevation of SOD in rat muscles, suggesting the involvement of XO-derived ROS in exercise adaptation through the activation of MAPK cascades (Gómez-Cabrera et al. 2005; Kang et al. 2009). Two studies (Ji et al. 2006; Radak et al. 2005) independently proposed that ROS-mediated adaptive responses in exercising muscle can be a biphasic dose-dependent process as low ROS doses exert beneficial effects, whereas high ROS doses are harmful (Gómez-Cabrera et al. 2005; Powers and Jackson 2008).

ROS-mediated muscle adaptations are present in both aerobic and anaerobic exercise training (Bogdanis 2012). The primary signaling pathway involves Nrf2, a redox-sensing transcription factor, which is responsible for the trans-activation of antioxidant genes. Nrf2 expression is upregulated during exercise. Mitochondrial biogenesis is another muscle adaption to exercise, since exercise training enhances mitochondrial biogenesis by upregulating PGC-1α expression. The activity of PGC-1α is regulated by upstream redox-sensitive signals such as MAPK and nuclear factor (NF)-κB (He et al. 2016). MAPK activates NF-κB and upregulates the expression of antioxidants, endothelial nitric oxide synthase (eNOS), and inducible NOS

(iNOS) (Gómez-Cabrera et al. 2005). eNOS and iNOS produce nitric oxide ($NO^{\bullet}$) and mediate vasodilation, which are essential for enhanced exercise capacity and attenuated I/R injury (Akita et al. 2007; Ji et al. 2007; Kojda et al. 2001). Together, elevations of $NO^{\bullet}$ and vascular endothelial growth factor (VEGF) lead to angiogenesis during exercise (Prior et al. 2004). Interestingly, the PGC-1α signaling pathway induces an alteration of phenotype from fast-twitch toward slow-twitch muscle, which increases fatigue resistance and enables continuous exercise (Lin et al. 2002; Zierath and Hawley 2004). Exercise-induced ROS could also play a role in protecting the brain by activating brain-derived neurotrophic factor (BDNF), which is involved in the regulation of various human activities including learning, locomotion, and stress responses (Radák et al. 2008). The expression of BDNF increases in response to oxidative stress, thus potentially initiating the protective effects in the brain, while exercise-induced VEGF expression also stimulates angiogenesis in the brain (Radák et al. 2008) (Fig. 10.1).

The beneficial effects offered by chronic exercise are potentially mediated by moderate increases in ROS during physical activities (He et al. 2016). Many studies have examined the potential favorable roles of ROS in chronic exercise. Increased Cu/Zn-SOD activity was observed in the whole aorta, while NOX activity was reduced in aortic endothelial cells in female Yucatan miniature pigs following chronic aerobic exercise during 16–19 weeks of aerobic training (Rush et al. 2003). Furthermore, there were reductions in the levels of MDA and ERK1/2 phosphorylation in the aorta of the exercised group (Rush et al. 2003). The decreased phosphorylation of ERK1/2 indicated lower oxidative stress induced by chronic aerobic training (Rush et al. 2003). These data suggested that long-term aerobic exercise attenuates oxidative stress in the aorta by enhancing the endogenous antioxidant defenses as well as decreasing the activity of prooxidant enzymes. Such changes are tightly coupled with improved cardiovascular health resulting from chronic aerobic exercise (Rush et al. 2003). Accordingly, Roque et al. reported similar results, showing that 12 weeks of treadmill training significantly improved $NO^{\bullet}$ bioavailability and decreased $O_2^{\bullet-}$ levels in the arteries of hypertensive rats (Roque et al. 2013). Experiments by Marosi et al. showed that low levels of ROS stimulated by chronic aerobic exercise lead to the activation of cascades involving calmodulin-dependent kinase (CaMKK)/AMP-activated protein kinase (AMPK)/PGC-1α (Marosi et al. 2012). It appears that long-term anaerobic training also leads to adaptation, yet the mechanisms remain elusive (Bloomer and Goldfarb 2004).

10.6 Antioxidants in Exercise

10.6.1 Aerobic Exercise and Endogenous Antioxidants

Multiple studies have shown that exposure to elevated ROS during regular exercise can strengthen the endogenous antioxidants in various key organs such as the skeletal muscle, liver, and brain (Fisher-Wellman and Bloomer 2009; Gomes et al. 2012; Radák et al. 2008). Further evidence indicates that the changes in antioxidant status

caused by aerobic exercise are tissue-dependent (Liu et al. 2000). Concentrations of vitamin C were significantly elevated in the rat brain following exhaustive treadmill running, while the levels remained constant in the liver and decreased in skeletal muscles (Liu et al. 2000). Radák et al. examined antioxidant activity in the hippocampus and cerebellum of rats after 6 days of strenuous treadmill running (6–8 min/day) and reported that exercise training at this intensity drastically increased plasma XO activities but had little effect on the redox status and antioxidant enzyme activities (Radák et al. 1995). Accordingly, it was later reported that the activities of various antioxidant enzymes such as SOD, CAT, and GPX were not significantly affected in the brain of rats following treadmill training (Radák et al. 2008). Although electron spin resonance revealed increased ROS levels in certain brain areas during exercise, there was no evidence of oxidative damage (Radák et al. 2008). It is likely that exercise-induced adaptations in the brain are region-specific (Morgan et al. 2015).

There are mixed results related to the regulatory effects of aerobic exercise on antioxidants in the skeletal muscle. For example, Lambertucci et al. investigated the alterations of antioxidant enzyme activities in the soleus from young and old rats following 13 weeks of moderate treadmill training. Exercise increased the activities of total SOD, Mn-SOD, CAT, and GPX, without changing Cu/Zn-SOD in young rats. However, in aged mice, the activities of those enzymes were either not affected or decreased (Lambertucci et al. 2007). Moreover, short-term treadmill exercises at moderate intensities enhanced the antioxidant capacities of SOD, CAT, and GSH in the diaphragm of rats. This upregulation of antioxidants was accompanied by reduced lipid peroxidation and attenuated fatigue development in diaphragm (Vincent et al. 2000).

Chronic aerobic activities markedly increased SOD activity, while the remaining aerobic exercise studies did not observe such alterations (Powers et al. 1999). The inconsistency of the results was attributed to the differences in the sensitivity of SOD assay techniques, changes in exercise protocols, as well as in the variety of muscle fiber type studied. Importantly, Power et al. suggested that the intensity of aerobic exercise dramatically impacts antioxidant activity. The activity of SOD is selectively upregulated in highly oxidative muscles such as red gastrocnemius and soleus (primarily Type IIA and Type I fibers). Accordingly, long-term aerobic exercises are associated with increased antioxidant enzyme activities (e.g., CAT and GPX) except for Cu/Zn-SOD (Lambertucci et al. 2007). Importantly, vigorous exercise significantly elevates plasma levels of vitamin C 2 h after training but caused no marked changes in vitamin E concentrations (Belviranlı and Gökbel 2006). Although it is well known that aerobic exercise contributes to increased antioxidant capacities, oxidative stress-induced tissue damage can still be observed following different types of aerobic exercise.

10.6.2 Anaerobic Exercise and Endogenous Antioxidants

It is suggested that anaerobic exercise potentially enhances antioxidant activity as an adaptive response to exercise-induced oxidative stress (Belviranlı and Gökbel 2006; Qiao et al. 2006). For instance, mice undergoing intermittent anaerobic swimming exercise for 6 consecutive days have an increased total antioxidant capacity

and higher levels of Cu/Zn-SOD in the heart and muscles, while elevated lipid peroxidation levels remained in the muscle but not in the brain (Qiao et al. 2006). These results suggest that the reduced exercise endurance caused by intermittent anaerobic activities may be linked with lipid peroxidation in the skeletal muscle, while intermittent anaerobic exercise can be beneficial in improving antioxidant activity in the central nervous system (Qiao et al. 2006). SOD activity is reduced immediately after high-intensity anaerobic exercise, but the activity of GPX did not change, while there were slight reductions in GSH levels following the exercise test (Groussard et al. 2003).

10.6.3 Antioxidant Supplementation

Considering the enhanced oxidative stress observed during anaerobic activities, an increasing number of studies have explored the potential effects of antioxidant supplementation on exercise performance (Slattery et al. 2014; Urso and Clarkson 2003). However, the delicate regulatory mechanisms of endogenous antioxidants have complicated the interpretation in the use of antioxidant supplementation. Findings from over 60 years of study still remain controversial regarding the use of antioxidants in attenuating the oxidative damage in tissues caused by exercise (Urso and Clarkson 2003).

10.6.3.1 Benefits of Antioxidant Supplements in Exercise

A protective effect of antioxidant supplements against oxidative damage elicited by exercise has been reported in several studies. For instance, in the study of Bloomer et al., aerobically trained subjects who have taken either vitamins (vitamin C and E) or fruits/vegetables for 2 weeks prior to exercise showed decreased protein carbonyl levels following 30-min running (Bloomer et al. 2006). Similar results were reported in which post-50 km ultramarathon elevation of F_2-IsoPs was suppressed by a 6-week pre-administration of vitamin C and E combination (Mastaloudis et al. 2004). Vitamins C and E are the most common dietary antioxidants to be investigated for their efficacy in improving physical performance. Vitamin E exerts protective effects on membrane structures (Gomes et al. 2012), while vitamin C alleviates muscle soreness (Staton 1952; Urso and Clarkson 2003). Muscle soreness observed after strenuous exercise can be a manifestation of muscle injury caused by overexercise (Urso and Clarkson 2003). Muscle damage-induced inflammatory process can lead to excessive oxidant production (Zuo et al. 2015a, b). Therefore, vitamin C can play a role in controlling the excessive free radical production, thus alleviating muscle soreness (Mastaloudis et al. 2006; Urso and Clarkson 2003). Until now, the use of vitamin supplementation on endurance exercise remains questionable as it is difficult to evaluate the benefits of these vitamins due to variables such as different methodologies, subject characteristics, and vitamin dosage (Draeger et al. 2014).

Studies that present the discordant results on vitamin supplements in exercise will be discussed in Sect. 10.6.3.2.

N-acetylcysteine (NAC) has been widely studied as a supplement during aerobic and anaerobic exercises. NAC is an acetylated cysteine residue and plays a critical role in maintaining GSH levels (Kerksick and Willoughby 2005). Dietary intake of NAC was recommended to athletes to prevent muscle damage after strenuous physical exercise in the 1990s (Gómez-Cabrera et al. 2015). NAC infusion improves force output in fatigued muscles, although it may have no effects on the non-fatigue muscle, suggesting that NAC enhances muscle fatigue resistance by mitigating oxidative stress induced by prolonged exercise (Kerksick and Willoughby 2005). In other studies, NAC was administrated via intravenous infusion to participants 45 min before and during exercise and that the time to fatigue at 92% VO_2 max was extended by NAC infusion in trained and fitter individuals (Medved et al. 2004a, b). The effects of NAC on long-term exercise performance could be dependent on the training status (VO_2 max) of the individual (Medved et al. 2004b). Importantly, the rise in plasma K^+ concentration at fatigue induced by exercise was attenuated by NAC (Medved et al. 2004b).

Alpha-lipoic acid (LA) was used to maintain cysteine levels (Kerksick and Willoughby 2005). Treatment with LA attenuates free radical increases and lipid peroxidation in plasma caused by strenuous aerobic exercise. The activities of GSH reductase and thioredoxin reductase are significantly elevated by LA supplementation (Kinnunen et al. 2009). These results suggest that LA can alleviate exercise-related oxidative stress by strengthening antioxidant defenses in the skeletal muscle (Chae et al. 2008; Kinnunen et al. 2009). The protective roles of LA were further confirmed by Chae et al. who reported that regular aerobic exercise combined with LA supplementation suppressed lipid peroxidation in the skeletal muscle (Chae et al. 2008).

Coenzyme Q10 (CoQ10) functions as antioxidant nutrient in a manner analogous to beta-carotene (vitamin A), alpha-tocopherol (vitamin E), and selenium (Zamora et al. 1991). CoQ10 is also an indispensable part of the mitochondrial electron transport chain, which provides the energy for muscle contraction (Crane 2001). Therefore, the use of CoQ10 as a dietary supplement has gained considerable attention for physical activity and sports performance. Intake of CoQ10 for 8 days improves fatigue resistance (Mizuno et al. 2008). A randomized, double-blind study was conducted to examine whether 8 weeks of CoQ10 supplementation could improve physical performance during repeated bouts of strenuous exercise (Gökbel et al. 2010). In this study, CoQ10 administration attenuated muscle fatigue during anaerobic exercise; thus, it has been proposed that CoQ10 is a possible ergogenic aid (physical performance enhancer) (Gökbel et al. 2010). Furthermore, CoQ10 has been linked with improved VO_2 max and anaerobic threshold (Peternelj and Coombes 2011).

Selenium (Se) is a cofactor of GPX and plays an important role in maintaining GSH-related antioxidant defenses (Clarkson and Thompson 2000; Sen and Packer 2000). Its effects on exercise-induced oxidative alterations and physical performance were evaluated (Clarkson and Thompson 2000). Three weeks of Se intake

reduces LOOH levels in overweight individuals after exercise (Savory et al. 2012). Se supplements prevented lipid peroxidation by promoting GSH activities (Akil et al. 2011). Worthy of note is that supplementation with the antioxidant, astaxanthin (1 mg/kg body weight; for 45 days), prior to exhaustive swimming in rats delays exhaustion time and also induces mitochondrial antioxidant responses. Thus, astaxanthin can enhance exercise performance by potentially reducing muscle oxidative stress (Polotow et al. 2014).

10.6.3.2 Potential Harms of Antioxidant Supplements in Exercise

Although antioxidants can protect tissues against oxidative damage during strenuous exercise, they can also negate ROS-mediated favorable adaptations to exercise. Excessive antioxidant intake can delay muscle strength restoration and the healing process after exhaustive training in athletes (He et al. 2016). Both human and animal studies indicate that the administration of vitamin C or E likely reduces exercise capacity and comprises exercise training efficiency (Fisher-Wellman and Bloomer 2009; Gómez-Cabrera et al. 2008a, b). For example, supplementation with vitamin A, vitamin E, and vitamin C interferes with the upregulation of heat-shock protein (HSP) 70, a protein that can enhance skeletal muscle performance following exercise (Niess and Simon 2007). A study by Connolly et al. found that 8 days of vitamin C supplementation (3000 mg/day) did not have protective effects against elbow flexor delayed onset muscle soreness (DOMS) (Connolly et al. 2006). These observations were associated with the inhibitory effects of vitamin C on IL-6 release from the skeletal muscle (Gómez-Cabrera et al. 2008a, b). IL-6, which can be stimulated by ROS during exercise, is a key regulator of muscle metabolism and facilitates the production of anti-inflammatory cytokines (Kosmidou et al. 2002; Pedersen and Fischer 2007). Therefore, vitamin C administration interrupts ROS-stimulated IL-6 release and hence diminishes its beneficial effects in maintaining muscle function.

Vitamin C supplementation (20 mg/kg) increased oxidative damage in the brains of mice after 6.5-week treadmill exercise, as evidenced by elevated levels of TBARS (Coşkun et al. 2005). Oral vitamin C exhibits prooxidant properties at high concentration or in the presence of iron and/or copper (Chakraborthy et al. 2014). Although vitamin C supplementation produced positive outcomes in improving anaerobic exercise performance (Urso and Clarkson 2003), other studies provide little convincing evidence that vitamin C improves anaerobic endurance (Keith and Merrill 1983; Urso and Clarkson 2003). There are also some reports that vitamin C can have negative effects on exercise performance and retard muscle recovery from exercise-induced oxidative damage (Gómez-Cabrera et al. 2008a, b; Marshall et al. 2002). Marshall et al. investigated that the racing speed of greyhounds when treated with vitamin C (1 g) daily in their food for 4 weeks ran an average of 0.2 s slower than the placebo groups. These results indicate that high doses of vitamin C compromise exercise performance (Marshall et al. 2002). In addition, high-dose vitamin C

impedes exercise-induced adaptation (Gómez-Cabrera et al. 2008a, b). In this study, placebo and vitamin C-supplemented groups of rats underwent 6 weeks of running exercise training. Prior to any training, rats could run 100 min before exhaustion. At the end of the training period, rats in the control group could run 300 min, while the rats treated with vitamin C could only run for 120 min (Gómez-Cabrera et al. 2008a, b). In this case, vitamin C abolished the benefits of ROS in mediating exercise adaptation (He et al. 2016).

Many studies also report limited or no significant protective effects of vitamin E on exercise-induced lipid peroxidation or benefits on exercise performance. This is likely attributed to the complex nature of vitamin E and its low accumulation rate in the muscle (Gomes et al. 2012; Stepanyan et al. 2014). Vitamin E supplementation leads to decreased exercise adaptive responses to ROS, as shown by Jackson et al. who concluded that increased muscle antioxidant content limits the upregulation of ROS and thus abolishes the subsequent transcription of adaptive genes during exercise (Jackson et al. 2004). One exception is exercising at a high altitude, where individuals are exposed to low O_2 and increased oxidative stress. Under these conditions, vitamin E intake improves exercise performance and reduces lipid peroxidation by scavenging ROS (Simon-Schnass and Pabst 1988; Urso and Clarkson 2003). In addition to antioxidant vitamins, there are potential side effects of NAC and CoQ10 administration. NAC hinders the activation of redox-sensitive AMPK and results in decreased glucose transport (Niess and Simon 2007). CoQ10 fails to provide ergogenic aid in human subjects (Zhou et al. 2005). Combined supplementation with NAC and vitamin C can exacerbate oxidative stress and inflammation during muscle damage (Niess and Simon 2007).

10.7 Conclusions

The complex role of ROS in exercise-induced oxidative stress and adaptation depends on the type, duration, and intensity of exercise. Physiological ROS levels are essential in facilitating muscle force generation, whereas excessive ROS can lead to oxidative damage and limited physical performance. Studies on antioxidant supplementation and muscle protection have yielded mixed results. The concepts of antioxidant supplementation for exercise require further investigation. The interactions of endogenous versus exogenous antioxidants, the doses of the antioxidant administration, the health of subjects involved, and the types and specificity of biomarkers should be given special attention. It is also imperative to establish standards for exercise testing protocols as well as developing reliable oxidative indicators when evaluating the effects of antioxidant administration in exercise performance and outcomes.

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Chapter 11
Plant Polyphenols in Healthcare and Aging

Kanti Bhooshan Pandey and Syed Ibrahim Rizvi

Abstract Aging is characterized by a progressive inability of organs of biological systems to defend against environmental stressors. Oxidative stress, a state of imbalance between cellular production of oxygen free radicals and reactive oxygen species (ROS) and their removal by antioxidants, has emerged as a critical player in aging process. Indeed, oxidative stress status is observed during aging and in numerous age-related diseases. The accumulation of deleterious oxidative damages occurring in cells with advancing age would induce damage of the vital cellular macromolecules, lipids, proteins, and DNA, which can potentially lead to cell dysfunction and death. Hence, organs and tissues accumulate free radical damage over time under conditions in which their endogenous antioxidant defenses are overwhelmed, resulting in overall cellular redox imbalance and impaired organ physiology. Dietary antioxidants are bioactive molecules, which can scavenge ROS and decrease the incidence of oxidative stress-induced damage. Plant antioxidants, including polyphenols, have been extensively studied for their beneficial health effects in human. There is evidence that populations consuming diets rich in polyphenols are less susceptible to oxidative damage and diseases during aging. The present chapter deals with the free radical theory of aging, providing current evidence of dietary interventions aimed at limiting the aging process. This chapter also describes the biological activities of some abundantly occurring polyphenols and their possible roles in healthcare as well as in prevention and treatment of age-related diseases.

Keywords Oxidative stress • Dietary antioxidants • Polyphenols • Healthy aging

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11.1 Introduction

The process of aging is characterized by a progressive inability of organs and tissues of biological systems to defend against environmental stressors that leads to alterations in the normal functioning of organism over time and an enhanced susceptibility to many diseases (Rattan 2006; Donato et al. 2015). The free radical theory of aging hypothesizes that oxygen free radicals generated during cellular metabolism and respiration are responsible for the age-related damage of organs and tissues in aerobic organisms (Harman 1956, 2006). Free radicals and other reactive oxygen species (ROS) damage mitochondrial DNA, which in turn induce mutations and alter mitochondrial function, including adenosine triphosphate (ATP) production (Sanz and Stefanatos 2008). Hence, cells accumulate free radical damage over time under conditions in which their antioxidant defenses are overwhelmed, resulting in overall cellular redox imbalance, organ dysfunction, and chronic diseases (Valko et al. 2007). Exhaustive research on the aging population repeatedly reports decrease in plasma antioxidant capacity with age (Pandey and Rizvi 2010b; Moreira et al. 2014).

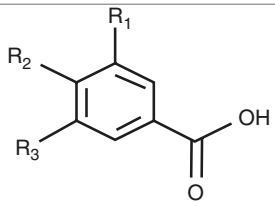
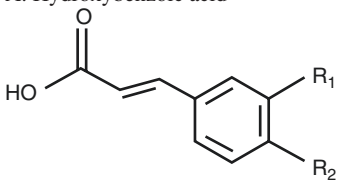
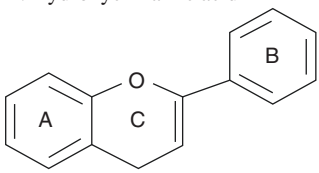
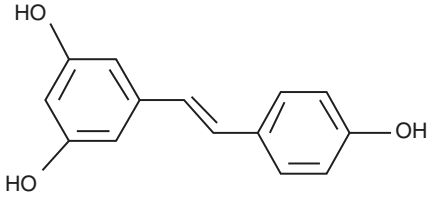
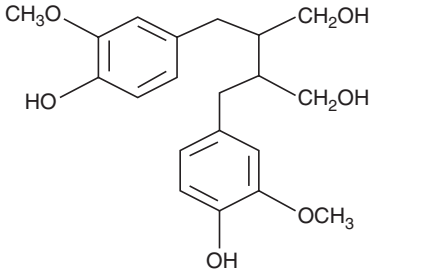
Natural antioxidants, including polyphenols, are abundantly present in many plants and their products. Polyphenols are synthesized in plants as secondary metabolites in times of adversity such as exposure to stressors: solar ultraviolet (UV) radiation, toxic heavy metals, and free radicals generated during the photosynthetic process or during pathogens attack (Stevenson and Hurst 2007). Polyphenols have been extensively studied for their beneficial pleiotropic biological effects in human health (Scalbert et al. 2005; Pandey and Rizvi 2009a; Moreira et al. 2014). Antioxidants are bioactive molecules, which can scavenge ROS and decrease the incidence of oxidative stress-induced cellular damage. Dietary supplementation with plant-derived polyphenols has emerged as a promising approach to counteract age-associated physiological dysfunction (Queen and Tollefsbol 2010). Clinical, nutritional, and epidemiological studies indicate that populations consuming diets rich in polyphenols are less susceptible to many age-associated pathologies such as cardiovascular disease (CVDs), diabetes, and cancer (Pandey and Rizvi 2009b; Khurana et al. 2013; Park and Pezzuto 2015). Considering that plants are major sources of various polyphenolic compounds, this chapter describes the biological activities of some abundantly occurring polyphenols and their possible roles in healthcare as well as in prevention and treatment of age-related chronic diseases.

11.2 Polyphenols: Occurrence, Types, and Structure

Polyphenols are the most numerous and widely distributed groups of molecules in the plant kingdom. More than eight thousand polyphenols have been identified in different food sources derived from plants (Pandey and Rizvi 2012a); however, their distribution is not uniform. Insoluble polyphenols such as resveratrol, myricetin, and astringin occur abundantly in cell walls, while soluble phenolics such as gallic

acid, cyanidin, and catechins are present in plant cell vacuoles. Fruits and vegetables are major sources of polyphenolic compounds. Linseed is a major source of lignans, where secoisolariciresinol levels can reach 3.7 g/kg dry weight (Adlercreutz and Mazur 1997). Blueberries, kiwis, cherries, and apples contain 0.5–2 g/kg of phenolic acids. Fresh onions have up to 1.2 mg/kg quercetin. Resveratrol content in red wine varies between 0.1 and 14 mg/L, while dried grape skins contain about 24.06 mg/g

Table 11.1 Different classes of plant polyphenols with their structure and examples

1. *Phenolic acids* Phenolic acids comprise about one third of total polyphenols known and are further divided into two subclasses: hydroxybenzoic acid and hydroxycinnamic acids. Phenolic acids generally do not exist in free form in nature but occur as glycosylated or ester derivatives Examples: gallic acid, protocatechuic acid, caffeic acid, and ferulic acid	 A. Hydroxybenzoic acid  B. Hydroxycinnamic acid
2. *Flavonoids* Flavonoids consist of two aromatic rings that are bound together by three carbon atoms that form an oxygenated heterocycle. Based on the variations in the type of heterocyclic involved, flavonoids are further divided into six subclasses Examples: quercetin, myricetin, catechins	 Divided in six subclasses: Flavonols, flavones, flavanones, flavanols, anthocyanins, isoflavones
3. *Stilbenes* Stilbenes contain two phenyl moieties connected by two-carbon methylene bridges. They are synthesized in response to infections or injury Examples: resveratrol, piceids, astringins	
4. *Lignans* Lignans are diphenolic compounds that contain a 2,3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues. Several lignans, such as secoisolariciresinol, are considered phytoestrogens Examples: secoisolariciresinol, matairesinol	

of resveratrol (Burns et al. 2002). Red wine and green tea contain up to 45 mg flavonoids/L (Pandey and Rizvi 2009c).

Although different polyphenols vary in their structures, they all stem from a common intermediate phenylalanine or a close precursor, shikimic acid (Spencer et al. 2008). Based on the number of phenol rings and the structural moieties that bind these rings to one another, polyphenols are classified into four major classes: phenolic acids, flavonoids, stilbenes, and lignans. Hydroxybenzoic acid and hydroxycinnamic acids are subclasses of phenolic acids since they are derived from two different precursors: benzoic acid and cinnamic acid. Flavonoids are further divided into six subclasses, flavonols, flavones, flavanones, flavanols, anthocyanins, and isoflavones (Table 11.1), on the basis of the type of heterocycle involved (Pandey and Rizvi 2009c).

11.3 Antiaging Effect of Plant Polyphenols

Polyphenols are reported to contribute to the human health benefits associated with consumption of diets rich in fruits and vegetables or plant-derived beverages. Plant polyphenols protect against oxidative damage and can extend life span in multiple species. Green tea polyphenols, mainly epigallocatechin gallate (EGCG) and epigallocatechin (EGC), have been reported to extend life span by almost 6% in the C57BL/6 mouse strain, possibly by mechanisms related to caloric restriction and hormesis (Kitani et al. 2007). Quercetin has also been reported to extend the life span of the nematode *Caenorhabditis elegans* (Saul et al. 2008). Quercetin is able to prevent the oxidation of lipids, proteins and DNA while also restoring the diminished antioxidant status in different types of cells such as erythrocytes and hepatic and endothelial cells in many organisms including humans (Pandey and Rizvi 2009b; Boots et al. 2008; Pandey et al. 2010). Likewise quercetin and caffeic acid can extend life span of nematodes by increasing antioxidative capacity in vivo and reducing oxidative damage (Pietsch et al. 2011). Population-based observational studies indicate that consumption of polyphenol-rich foods reduced mortality rates and the incidence of CVDs and cancer (Stevenson and Hurst 2007). Other evidence supporting the antiaging effect of polyphenols is the fact that consumption of red wine (Baur and Sinclair 2006) or green tea (Khan and Mukhtar 2013) is associated with reduced rates of mortality. Furthermore, polyphenols have also the potential to counteract age-associated diseases including cancer, based on their ability to modulate master regulatory molecules involved in various disease states (Novelle et al. 2015).

11.4 Antioxidant Effect of Specific Plant Polyphenols

Epidemiological studies report that dietary polyphenols enhance plasma antioxidant capacity and protect biological systems from oxidative injury during the aging process (Khurana et al. 2013, Singh and Rizvi 2015). Importantly, the antioxidant

effect of specific plant polyphenols, mainly catechins, curcumin, and resveratrol, was the subject of considerable studies.

11.4.1 Catechins

Green tea catechins are the most widely studied natural polyphenols, particularly because of their pleiotropic biological effects (Khan and Mukhtar 2013). Rats receiving green tea extract exhibit higher levels of antioxidant enzymes including glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) (Skrzydlewska et al. 2005). A study in humans demonstrated that consumption of two cups of green tea containing about 250 mg of total catechins for 42 days significantly improved plasma total antioxidants while also reducing peroxide levels (Singh et al. 2008). Catechins decrease the impact of oxidative burden by inhibiting the expression of xanthine oxidase, a key enzyme for the generation of free radicals in the body (Bickford et al. 2000). Catechins protect the oxidation of lipids in the aging brain and liver (Skrzydlewska et al. 2005). Various mechanisms have been proposed through which catechins elicit antioxidant activities including chelation of metal ions such as Fe (II) and Cu (II). Ultra-rapid transfer of electrons to ROS-induced radical sites on DNA is another mechanism by which catechins can act as antioxidants (Singh et al. 2008). Moreover, oxidation of catechins by ROS results in a dimerized product, which has the ability to scavenge superoxide ($^{\bullet}O^{2-}$) free radicals (Singh et al. 2008).

11.4.2 Curcumin

Curcumin in micro molar concentration scavenges ROS in both in vitro and in vivo studies (Joe and Lokesh 1994; Singh and Rizvi 2015). Altered ion homeostasis and enhanced osmotic fragility, which is an index of membrane integrity, have been reported during aging in the study carried out on 91 normal healthy subjects of both sexes (59 males and 32 females) between the ages of 18 and 80 years (Pandey and Rizvi 2013, 2014a, b). Glutathione plays an important role in antioxidant defenses either by reacting directly with ROS or by acting as cofactor for GPx. Curcumin has the potential to reduce myocardial lipid peroxidation by increasing glutathione content and GPx activity (Venkatesan 1998). Experimental studies using several cell models document that curcumin protects the cellular integrity and homeostasis by preventing the peroxidation of lipids and oxidation of proteins (Kolodziejczyk et al. 2011). It is important to note that treatment with 0.1–10 μM curcumin reduces the intracellular ROS levels in many types of cells during adverse conditions such as inflammation, oxidative stress, and neuronal disorders (Singh et al. 2008; Kim et al. 2007; Singh and Rizvi 2015).

11.4.3 Resveratrol

Resveratrol, a natural compound with anti-inflammatory and antioxidant properties (Bo et al. 2013), has been reported to prevent CVDs, diabetes mellitus, and neurodegenerative disorders (Smoliga et al. 2011). Significant increases in plasma antioxidant level and reductions in lipid peroxidation occur after consumption of resveratrol-rich diets (Wenzel et al. 2005). Oxidation of low-density lipoprotein (LDL) particles is strongly associated with the risk of developing CVDs and myocardial infarction during aging (Baur and Sinclair 2006). Resveratrol prevents oxidation of LDL and hence provides protection against myocardial infarction (Urpí-Sardà et al. 2005). Resveratrol reduces glycated albumin in serum and 8-hydroxyguanosine in urine, both markers of oxidative stress, in stroke-prone spontaneously hypersensitive rats (Mizutani et al. 2001). Resveratrol (10–100 μM) decreases the expression of the superoxide-producing enzyme NADPH oxidase 4 (Nox4) and, at the same time, increases protein expression of ROS scavenging antioxidant enzymes, SOD 1 and GPx, in human endothelial cells (Spanier et al. 2009). It has been also reported that resveratrol provides protection against lipid peroxidation, protein carbonylation, and sulfhydryl group oxidation in erythrocytes during aging in humans (Pandey and Rizvi 2013).

11.5 Activation of Plasma Membrane Redox System by Polyphenols

The impairment of cellular homeostasis during aging is well described (Radak et al. 2011; Sohal and Orr 2012). There is a mounting realization that the structural and functional damage-based hypothesis of aging process actually involves a shift in cellular redox states (Sohal and Orr 2012; Rizvi and Jha 2011). A group of oxidoreductase enzymes known as plasma membrane redox system (PMRS) exist in the plasma membrane of the eukaryotic cells; these enzymes play a crucial role in maintaining the redox state of the cell (Hyun et al. 2006; Pandey and Rizvi 2010a). The PMRS transfer reducing equivalents from intracellular donors such as ascorbate and nicotinamide adenine dinucleotide reduced (NADH) to extracellular acceptors that are used to maintain homeostasis though several mechanisms (Rizvi et al. 2009; VanDuijn et al. 1998).

The PMRS along with the enzyme ascorbate free radical (AFR) reductase recycles ascorbate in the plasma. The role of PMRS becomes very significant since ascorbate acts as a primary antioxidant in the body and also serves as a cofactor in many important enzymatic reactions (Harrison and May 2009). Activation of the PMRS along with AFR reductase is hypothesized to be a compensatory mechanism operating in the body to minimize the redox imbalance that occurs during aging (Pandey and Rizvi 2012a; Rizvi et al. 2009). An enhancement of PMRS is a mechanism by which caloric restriction may counteract mitochondrial dysfunction and

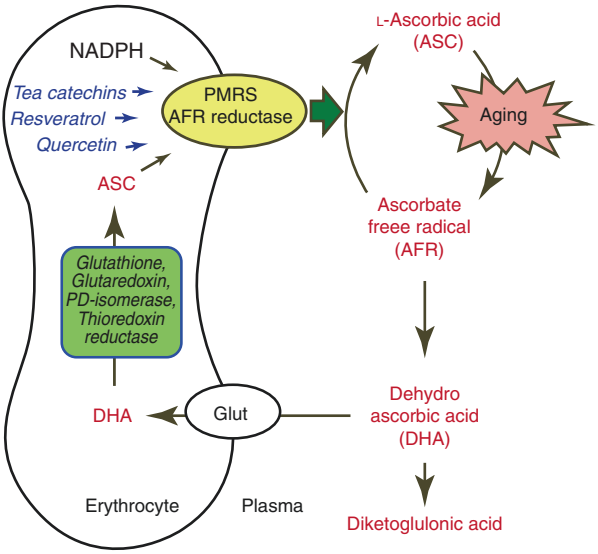


Fig. 11.1 Schematic representation of the role of polyphenols in maintaining intra- and extracellular redox states in human erythrocytes. *PMRS* plasma membrane redox system, *NADH* nicotinamide adenine dinucleotide, *Glut* glucose transporter

oxidative stress in the brain during aging (Hyun et al. 2006). Upregulation of PMRS is considered as an effective antiaging strategy (Rizvi and Jha 2011). Many polyphenols can activate the PMRS and AFR reductase in human cells. A study of 97 human male and female subjects aged between 18 and 82 documents that resveratrol significantly upregulates the redox system and ascorbate recycling during aging (Pandey and Rizvi 2013). It has been proposed that some polyphenols can enter in cells and, once inside, accumulate in higher concentrations compared to their concentration in the plasma. Quercetin and green tea catechins including EGCG and EGC activate the PMRS by accumulating in cells and donating electrons within the intracellular compartment in a dose-dependent manner (Fig. 11.1) (Pandey and Rizvi 2012b; Rizvi et al. 2010).

11.6 Caloric-Restriction Mimicking Effect of Polyphenols

Caloric restriction is a reduction in food intake without malnutrition. Studies report that 2–30% reduced caloric intake prolongs life span of various organisms, including yeast, nematodes, rodents, and some nonhuman primates (Colman et al. 2009, 2014; Fontana et al. 2010; Mercken et al. 2012). Caloric restriction has been reported to extend the average and/or maximum life span in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila*, mice, and rhesus monkeys (Fontana et al. 2010). In both rodents and monkeys, caloric restriction also delays loss of function

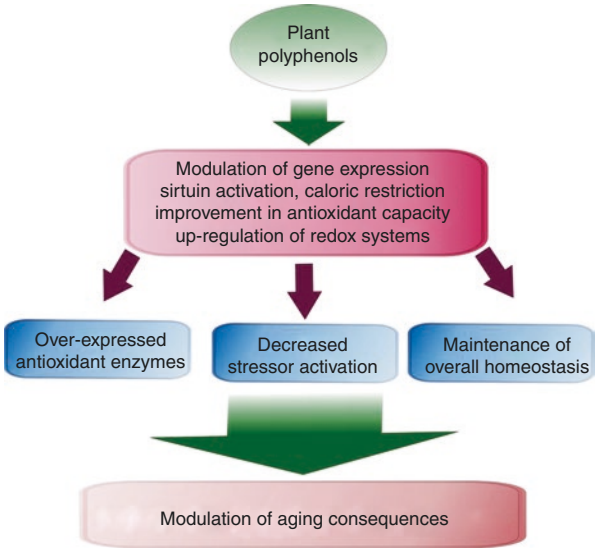


Fig. 11.2 Diagrammatic representation of role of polyphenols in mitigating aging consequences

and reduces the incidence of chronic diseases, and in humans it causes a reduction in metabolic markers of many diseases such as diabetes, neural problems, cancer, and CVDs (Omodei and Fontana 2011). Recent studies on aging interventions suggest that consumption of polyphenols mimics caloric restriction and may thus mitigate age-dependent diseases (Fig. 11.2).

Studies on resveratrol indicate that the caloric restriction mimicking effect of this stilbene that may underlie its role in extending longevity does so without reducing fecundity in lower organisms (Howitz et al. 2003) or in short-lived invertebrates such as the fruit fly *Drosophila melanogaster* (Wood et al. 2004; Bhullar and Hubbard 2015). Mean life span was extended by up to 70%, 18%, and 29% on treatment of resveratrol in lower organisms, short-lived invertebrates, and fruit flies, respectively. This effect has been attributed to activation of the sirtuin (SIRT) class of nicotinamide adenine dinucleotide (NAD)-dependent deacetylases. Seven sirtuins have been identified in mammals, of which SIRT-1 is believed to mediate the beneficial effects on health and longevity of both caloric restriction and resveratrol (Soleas et al. 2001; Orallo 2008).

Resveratrol prolongs mean life span of the short-lived seasonal fish *Nothobranchius furzeri* by up to approximately 56% (Valenzano et al. 2006), retards the expression of age-dependent traits by delaying the age-dependent decay of cognitive performances, and reduces the expression of neurofibrillary degeneration in the brains of *Nothobranchius furzeri* (Valenzano et al. 2006). Resveratrol promotes longevity and improves glucose homeostasis and energy balance and increases mitochondrial function in mice fed with a high-fat diet by stimulating the SIRT1-mediated deacetylation of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (Lagouge et al. 2006; Baur et al. 2006). PGC-1α is a key regulator of mitochondria; the targets of its

co-activation are the nuclear receptor family of transcription factors that are involved in multiple aspects of metabolism (Anderson and Weindruch 2012). Glucose restriction triggers 5′ adenosine monophosphate-activated protein kinase (AMPK) activity and activates the gene encoding the NAD synthetic enzyme, Nampt, which is necessary for the activation of the SIRT1. AMPK is also a PGC-1α activator. Many polyphenols modulate kinase pathways including AMPK and thus simultaneously modulate redox signaling and inhibit mitochondrial function (Joven et al. 2014). Therefore, a reduction in stress signaling and a subsequent reduction in ATP production may be predictable outcomes of polyphenol ingestion that suggest important implications for healthy aging.

11.7 Anti-inflammatory Effects of Polyphenols

Inflammation is considered as paramount to chronic disease development in modern lifestyle contributing to most age-related chronic diseases including CVDs, cancer, and Alzheimer disease (Joseph et al. 2016). Many in vitro and in vivo studies suggest that certain polyphenols possess anti-inflammatory properties that may be linked with the preventive effect of these secondary metabolites on onset, progression, and complications of inflammatory diseases. Resveratrol may act in vivo as an anti-inflammatory agent by inhibiting inflammatory cytokine expression in response to the lipopolysaccharides in lungs of rats (Birrell et al. 2005). Furthermore, resveratrol treatment decreases the overexpression of both vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 by inhibiting the nuclear factor-kappa B (NF-kB) pathway in tumor necrosis factor alpha (TNFα)-activated endothelial cells (Deng et al. 2011). Resveratrol is proposed to prevent chronic inflammation during obesity through reducing pro-inflammatory cytokine secretion and enhancing adiponectin release from human adipose tissue (Olholm et al. 2010).

Although several mechanisms have been proposed to explain the anti-inflammatory effect of quercetin, primarily it is the activation of signaling pathways involved in NF-kB activation which is considered to be the most important. Quercetin at 10 μM concentration is reported to decrease mRNA and protein levels of TNFα, interleukin (IL)-1β, IL-6, macrophage inflammatory protein-1α, and inducible nitric oxide synthase (iNOS) in various studies (Boesch-Saadatmandi et al. 2011; Andriantsitohaina et al. 2012). Quercetin has pleiotropic effects in apoE-KO mice including reduction of pro-inflammatory markers and enhancement of anti-inflammatory indicators such as endothelial NOS (eNOS) and heme oxygenase-1 expression, suggesting that quercetin at a dose of 64 mg/kg body mass daily could delay the process of atherosclerosis through its anti-inflammatory properties (Loke et al. 2010).

Delphinidin, one of the most studied anthocyanins, has been found to exhibit anti-inflammatory effect by directly interacting with kinases (Kwon et al. 2009). In mouse epidermal cells, delphinidin at concentration of 5–20 μM suppresses cyclooxygenase (COX)-2 promoter activity and COX-2 expression by inhibiting activa-

tor protein-1 and NF-kB pathways. The study reports that these effects shown by delphinidin are a result of the direct binding of this polyphenol to the ATP-binding site in the kinase domain of mitogen-activated protein kinase 4 and to the ATP-binding site of the catalytic domain of phosphatidylinositol-3-kinase (Kwon et al. 2009; Andriantsitohaina et al. 2012). Curcumin has significant anti-inflammatory activity, as reported in studies showing that it can inhibit lipoxygenase and COX-2, suppress iNOS levels, and act as a potent inhibitor of NF-kB (Bengmark 2006).

There is evidence in support for an anti-inflammatory effect of green tea extract, possibly mediated by the ability to scavenge nitric oxide ($NO^{\bullet}$) and peroxynitrite anion (ONOO–) and to inhibit the expression of iNOS (Tedeschi et al. 2004). The iNOS enzyme is expressed in organs including the lungs and intestine, where an overproduction of $NO^{\bullet}$ contributes significantly to many chronic diseases such as inflammatory bowel disease, celiac disease, Crohn's disease, asthma, vascular failure, and end-organ damage during endotoxemia and septic shock (Guslandi 1998; Tedeschi et al. 2004). In aging brain catechins inhibit the expression of neuronal NOS. This effect of catechins is likely to be involved in the suppression of the activation of transcription factor NF-κB as the κB sequence is present in the promoter region of the iNOS gene (Lin and Lin 1997; Chan et al. 1997). Catechins could induce the endothelial isoform eNOS to elicit anti-inflammatory effects (Lorenz et al. 2004; Singh et al. 2008). EGCG acts as anti-inflammatory agent by inhibiting the production of IL-1 and attenuating the IL-1-induced expression of COX-2 (Kim et al. 2007).

11.8 Dietary Polyphenols and Endothelial Function

Endothelial dysfunction is an important impairment during aging. Age-associated progressive induction of an endothelial dysfunction in arteries promotes the initiation and development of CVDs during aging (Matz et al. 2000). Endothelial cells play a key role in the regulation of vascular homeostasis in several ways including through the release of potent vaso-protective factors such as $NO^{\bullet}$, prostacyclin (PGI2), and endothelium-derived hyperpolarizing factor (EDHF) (Andriantsitohaina et al. 2012). There is substantial evidence that many polyphenols provide significant vascular protection against progression as well as development of CVDs (Andriantsitohaina et al. 2012; Bollmann et al. 2014). The polyphenol-mediated endothelium-dependent relaxations were first reported by Fitzpatrick and colleagues in 1993, which showed that grape skin extract and grape juice cause endothelium-dependent relaxations in aortic rings; however, direct evidence that polyphenols stimulate endothelial $NO^{\bullet}$ formation was documented by using electron paramagnetic resonance spectroscopy using aortic rings and cultured endothelial cells of rats (Fitzpatrick et al. 1993). There is evidence that red wine polyphenols (3 μg/ml) induce the endothelium-dependent relaxation in porcine coronary artery rings (Ndiaye et al. 2005).

Daily consumption of 10 mg resveratrol for 3 months improves endothelial function as measured by flow-mediated vasodilation in patients with stable coronary artery disease (Magyar et al. 2012). One-month resveratrol treatment (400 mg per day) on endothelial response reduces mRNA levels of inflammatory and adhesion molecule markers commonly associated with atherosclerosis (Agarwal et al. 2013). Resveratrol stimulates eNOS activity to enhance endothelium-dependent vasodilation (Wallerath 2002). The selective inhibition of COX-1 over COX-2 reduces endothelial inflammation and platelet aggregation (Baur and Sinclair 2006). Endothelial dysfunction in porcine coronary arteries due to homocysteine-induced impairment of endothelium-dependent vasorelaxation was reversed by curcumin, possibly by increasing eNOS levels as well as reducing $^{\bullet}O^{2-}$ production (Ramaswami et al. 2004). Curcumin mitigates accelerated aging after irradiation in *Drosophila melanogaster* by reducing oxidative stress (Seong et al. 2015). The study demonstrates that pretreatment with 100 μM curcumin recovered the irradiation-mediated shortened life span of *D. melanogaster*. It has been suggested that vascular protective effect of quercetin is associated with eNOS upregulation (Kukongviriyapan et al. 2012). Studies on rat aortic ring segments show that quercetin treatment for half an hour enhanced relaxation of aortic rings by virtue of NOS and endothelium-derived hyperpolarizing factor. Another study performed by the same group demonstrated that bovine aortic endothelial cells, when incubated with quercetin, exhibited an increase in intracellular calcium, eNOS phosphorylation, and subsequent increase in $NO^{\bullet}$ (Chirumbolo 2012). Taken together, all these results suggest that quercetin-induced phosphorylation of eNOS can increase availability of $NO^{\bullet}$, thereby inducing protective vascular effects during aging.

11.9 Conclusions

The overall decline in organ function plays a crucial role in aging and age-associated diseases. Oxidative stress, a state of imbalance between cellular production ROS and their removal by antioxidants, has emerged as a critical player in aging process. Oxidative stress status is observed during aging and in numerous age-related diseases. Hence organs and tissues accumulate ROS damage over time under conditions in which their endogenous antioxidant defenses are overwhelmed, resulting in overall cellular redox imbalance and impaired organ physiology. Plant polyphenols can scavenge ROS and decrease the incidence of oxidative stress-induced damage. There is evidence that populations consuming diets rich in polyphenols are less susceptible to oxidative damage and diseases during aging.

Plant polyphenols have demonstrated potential against progression of many age-associated pathologies in laboratory animal and epidemiological studies through different mechanisms. There is evidence to suggest that dietary polyphenols such as resveratrol, EGCG, and curcumin have the capacity to mitigate age-associated cellular damage induced by excessive ROS production. Caloric restriction mimicking

effect and enhancement of life span are hotly investigated biological activities of some polyphenols. Despite certain gray areas linking consumption of polyphenols and purported healthy and antiaging benefits, the present chapter provides a current understanding of the role of polyphenols in aging and age-associated chronic diseases.

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Chapter 12
Free Radicals and Antioxidants in Human Disease

Michael Lawson, Klaudia Jomova, Patrik Poprac, Kamil Kuča, Kamil Musílek, and Marian Valko

Abstract Free radicals are species containing one or more unpaired electrons. Unpaired or free electrons are responsible for enhanced reactivity of free radicals with various biomolecules. Most frequently occurring radicals in biological systems are reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are generated by the tightly regulated enzymes, nicotinamide adenine dinucleotide phosphate oxidase isoforms and nitric oxide synthases. Overproduction of ROS and RNS results in oxidative and nitrosative stress, a state which is responsible for the damage to cell macromolecules including lipids, proteins and DNA. Oxidative stress has been implicated in the aetiology of various disease states of an organism. In this chapter, we discuss the biochemistry of free radicals and their impact on the development of various diseases. Organs of biological systems are the principal targets of oxidant species, which are implicated in atherosclerosis, diabetes, carcinogenesis and neurodegeneration. Attention is focused on oxidative stress-induced cardiovascular disease, type 2 diabetes, cancer and Alzheimer's disease. The roles of redox active metal-catalysed formation of ROS and antioxidants in protection against oxidative damage is also discussed.

Keywords Free radicals • Oxidative stress • Antioxidants • Human disease

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12.1 Introduction

The effect of reactive oxygen species (ROS) and reactive nitrogen species (RNS) causing potential biological damage is termed oxidative stress and nitrosative stress, respectively (Kovacic and Jacintho 2001; Ridnour et al. 2005; Valko et al. 2004). This state is typical for overproduction of ROS/RNS on one hand and/or a deficiency of antioxidants on the other hand. Mild oxidative stress on cells may lead to apoptosis and cells are able to overcome it by regulating the redox state. However, enhanced and prolonged oxidative stress can cause necrosis. Long-term effects of oxidative stress cause damage to cellular lipids, proteins and DNA. DNA damage by free radicals leads to formation of a variety of oxidative products. One of the most extensively studied single-base damages by oxidation is 8-hydroxy-2′-deoxyguanosine (8-oxodG) (Dizdaroglu et al. 2002). The level of 8-oxodG in urine can be regarded as a sensitive marker of oxidative stress of an organism.

Organs of biological systems are the principal targets of oxidant species, which are implicated in atherosclerosis, diabetes, carcinogenesis and neurodegeneration. Cardiovascular disease CVDs is a problem in the Western developed world. It is generally believed to be a problem of people who have a high-fat diet and inadequate regular physical exercise. However, some individuals have a genetic preposition and the disease is more common in middle age and beyond. The latter suggests that free radical damage may play a role. ROS-induced oxidative stress is believed to play some role in the cardiovascular diseases such as atherosclerosis, ischemic heart disease, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure (Kukreja and Hess 1992). Type 2 diabetes is generally recognized as a polygenic disease. It is believed that it develops due to a cascade of events but that the most important of these events include oxidative stress-related defects in oxidative phosphorylation machinery and mitochondrial β-oxidation leading to accumulation of intracellular triglyceride in the muscle and liver and subsequent insulin resistance (Rosca et al. 2005). β-Oxidation of long-chain fatty acids is particularly important for provision of energy in the cardiac and skeletal muscle. Changes in the expression and function of the mitochondrial inner membrane protein—uncoupling protein-2 (UCP-2)—may play an important role in pancreatic β-cell dysfunction (Krauss et al. 2003). Disturbed redox regulation as a consequence of enhanced oxidative stress has been found in various cancer cells (Milkovic et al. 2014). DNA modifications by species produced by oxidative stress mechanisms represent the first step in carcinogenesis. Mutations of genetic material appear to be a critical point not only in carcinogenesis but also in ageing. Many tumours of various origins revealed increased DNA lesions compared to normal tissues. DNA damage induced by a variety of free radicals leads to formation of more than 150 oxidized products which can now be quantified with a high level of reproducibility (Dizdaroglu et al. 2002). The most frequent modification of genetic material involves single- to double-strand breaks and pyrimidine or purine modifications which in turn may affect signalling pathways, replication errors and overall genomic instability, all forming a common denominator of cancer (Marnett 2000). The studies of Alzheimer's

disease (AD) have been directed to amyloid beta protein (Aβ) (Ow and Dunstan 2014). Monomeric Aβ is a peptide with antioxidant activity and is the main constituent of amyloid plaques in the brains of AD patients. Increased formation of Aβ linked with enhanced oxidative stress and neurotoxicity is postulated to represent a major event in the development of AD.

In this chapter, we discuss the biochemistry of free radicals and their impact on the development of CVDs, cancer, type 2 diabetes and AD. The involvement of ROS/NOS appears to be the common denominator to all these events. The roles of redox active metal-catalysed formation of ROS and antioxidants in protection against oxidative damage are also discussed. An integrated view of the sources of ROS, ROS-induced damage to main biomolecules and disease incidence is outlined in Fig. 12.1.

12.2 Free Radicals, Antioxidants and Oxidative Stress

Free radicals are species containing one or more unpaired electrons. Unpaired or free electrons are responsible for enhanced reactivity of free radicals with various biomolecules. The superoxide anion ($^{\bullet}O_2^-$) and nitric oxide (NO) are the most frequently occurring radicals in biological systems (Valko et al. 2007). Radicals derived from oxygen can be considered as the most important class of species formed in living systems. Molecular dioxygen has a specific electronic configuration; in the ground state it contains two parallel unpaired electrons in antibonding π^* orbital (Halliwell and Gutteridge 1990). Thus molecular dioxygen is a biradical with electron spin quantum number $S = 1$. Thanks to the parallel orientation of both spins of electrons, reactivity of oxygen with biomolecules (having chemical bonds formed by two electrons with antiparallel spins) is significantly reduced (Valko et al. 2004). In addition to triplet molecular dioxygen, there also exist two singlet states. In singlet states, both unpaired electrons on antibonding π^* orbitals are antiparallel. In one of the oxygen singlet states, the two antiparallel electrons are localized on different orbitals. The lifetime of such oxygen is short and very rapidly interconverts to another form of singlet oxygen ($^1O_2$), also with two antiparallel electron spins, but located on the same orbital. $^1O_2$ is the most relevant source of singlet molecular oxygen in biological systems (Cadet et al. 2000).

Addition of an electron to molecular dioxygen leads to formation of superoxide radical ($^{\bullet}O_2^-$). The added electron fills one of the half-occupied antibonding π^* orbitals. $^{\bullet}O_2^-$ is considered as a primary radical formed in various biological processes. $^{\bullet}O_2^-$ is formed by four single-electron reduction steps from molecular dioxygen into water. In addition, the major source of $^{\bullet}O_2^-$ generated in aerobic cells is via electron leakage that occurs from electron transport chains including mitochondria and the endoplasmic reticulum. $^{\bullet}O_2^-$ is produced by activated phagocytes and neutrophils in order to kill bacteria. $^{\bullet}O_2^-$ can undergo a dismutation reaction catalysed

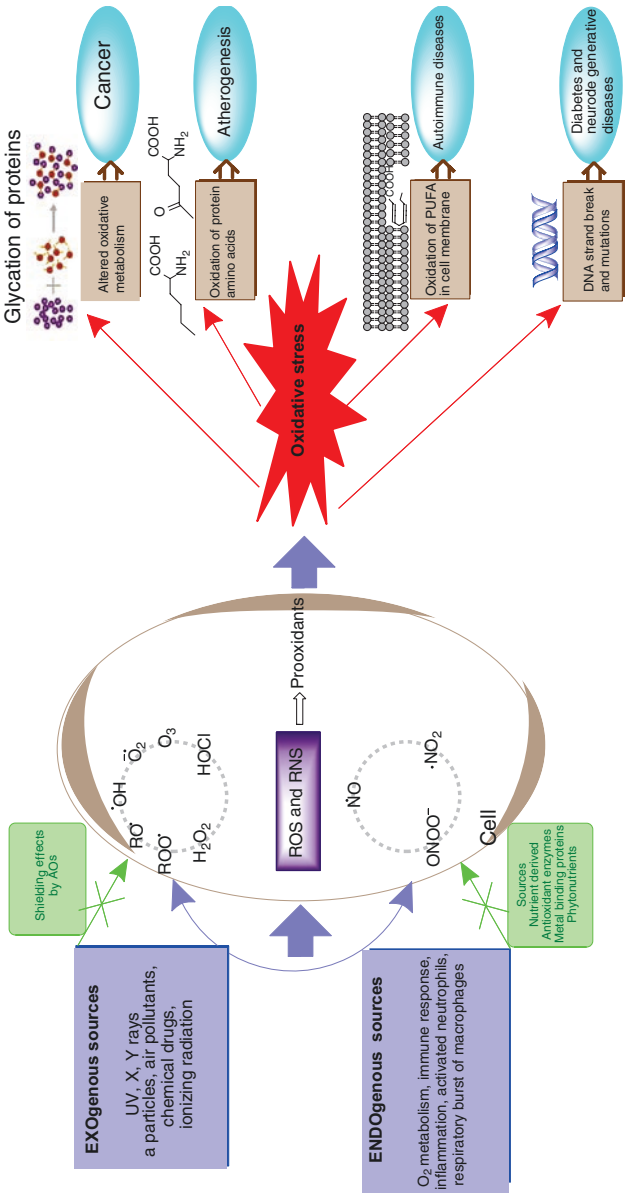

Fig. 12.1 A schematic view of the sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and associated oxidative damage to biomolecules and incidence of chronic diseases

by the enzyme superoxide dismutase (SOD) according to the reaction (Gutteridge et al. 1982):

$$2^{\bullet}O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

We note that the SOD enzyme works in conjunction with enzymes which remove hydrogen peroxide (H_2O_2) such as catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR).

The hydroxyl radical ($^{\bullet}OH$) is a very reactive species causing damage to all types of biomolecules. One way to generate $^{\bullet}OH$ in biological systems is via a metal-catalysed Haber-Weiss reaction (Enami et al. 2014):

$$^{\bullet}O_2^- + H_2O_2 \xrightarrow{Fe^{2+}} O_2 + {}^{\bullet}OH + OH^-$$

The Haber-Weiss reaction is an overall reaction consisting of two reactions:

$$Fe^{3+} + {}^{\bullet}O_2^- \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$$

The second reaction is the well-known Fenton reaction considered as a major source of $^{\bullet}OH$ in biological systems. $^{\bullet}OH$ is highly reactive with a very short half-life in aqueous solution (<1 ns). Under in vivo conditions, formation of $^{\bullet}OH$ in the proximity of important biomolecules may cause damage to them. Formation of $^{\bullet}OH$ close to DNA may cause damage to DNA bases or deoxyribosyl backbone of DNA.

The major source of $^{\bullet}O_2^-$ is from the mitochondrial electron transport chain. Ubisemiquinone is the main reductant agent of oxygen in mitochondrial membranes (Inoue et al. 2003). Mitochondria are able to produce ~2–3 nmol of $^{\bullet}O_2^-$/min per mg of protein, confirming mitochondria to be the most important physiological source of $^{\bullet}O_2^-$ in living organisms (Inoue et al. 2003). Mitochondria are highly abundant with antioxidants including the reduced glutathione (GSH), manganese SOD (Mn-SOD) and GPX, which are present on both sides of their membranes to suppress oxidative stress in the organelle (Cadenas and Davies 2000). Since $^{\bullet}O_2^-$ is negatively charged, it tends to stay in the proximity of the inner mitochondrial compartment where conversion into H_2O_2 by Mn-SOD enzyme may take place. H_2O_2 may diffuse through mitochondrial membrane (Flynn and Melov 2013) and if not eliminated (e.g. by CAT) may act as a substrate in the metal-catalysed decomposition (Fenton reaction) forming $^{\bullet}OH$. However, under physiological conditions, formation of $^{\bullet}O_2^-$ and H_2O_2 is thermodynamically rather unfavourable, and therefore we cannot expect a significant increase in $^{\bullet}O_2^-$ and H_2O_2 formation. A different situation may occur under pathological conditions, under which more profound formation of these species can be expected.

In addition to mitochondria, there exists other cellular sources of $^{\bullet}O_2^-$, including xanthine oxidase (XO) (Stein and Kirk 2015). XO is a highly versatile enzyme and

an important source of ROS. XO catalyses the reaction of hypoxanthine to xanthine and xanthine to uric acid. Additional endogenous cellular sources of ROS are neutrophils, eosinophils and macrophages. Cytochrome P450 is another source of ROS, in particular $^{\bullet}O_2^-$ and H_2O_2. Further sources of H_2O_2 include microsomes and peroxisomes.

Besides ROS, RNS play key roles in various biological processes (Gruetter et al. 1980; Bogdan 2015). Nitric oxide ($NO^{\bullet}$) contains one unpaired electron and therefore is also a radical. $NO^{\bullet}$ is an important signalling molecule in a variety of physiological processes, including regulation of blood pressure, defence mechanisms, smooth muscle relaxation and immune and neurological regulation (Ghafourifar and Cadenas 2005). $NO^{\bullet}$ is generated in biological tissues by specific nitric oxide synthases (NOSs). Overproduction of RNS is called nitrosative stress (Giuffrè et al. 2014). This may occur when the generation of RNS in a system exceeds the system's ability to neutralize and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function. Cells of the immune system produce both the $^{\bullet}O_2^-$ and $NO^{\bullet}$ during the oxidative burst triggered during inflammatory processes. Under these conditions, $NO^{\bullet}$ and $^{\bullet}O_2^-$ may react together forming the very reactive peroxynitrite anion ($ONOO^-$) (Carr et al. 2000):

$$NO^{\bullet} + {}^{\bullet}O_2^- \rightarrow ONOO^-$$

This reaction proceeds very fast and the product of the reaction can cause DNA damage and peroxidation of lipids (Carr et al. 2000). The harmful effect of ROS and RNS is suppressed by the action of enzymatic antioxidants as well as by small molecular weight antioxidants. The most efficient antioxidant enzymes involve SOD, CAT and GPX. SOD exists in several isoforms, cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD and extracellular SOD (EC-SOD) (Landis and Tower 2005). As already discussed above, SOD converts $^{\bullet}O_2^-$ into H_2O_2 at the enzyme active site in a "ping-pong"-type mechanism (McCord and Fridovich 2014). A different SOD enzyme that contains nickel (Ni-SOD) containing 117 amino acids was recently discovered in *Streptomyces* and cyanobacteria (Shearer and Long 2006; Shearer 2014). CAT is an enzyme located in a cell organelle called the peroxisome (Njuma et al. 2014). The enzyme is very efficient in the decomposition of H_2O_2 into water and molecular oxygen. It has been estimated that one molecule of CAT converts approximately six million molecules of H_2O_2 into water and oxygen. In various types of tumours, suppressed capacity of CAT to detoxify H_2O_2 was observed. GSH metabolism belongs to one of the most effective antioxidative defence mechanisms. There are two forms, selenium-independent glutathione *S*-transferases (GST) and selenium-dependent GPXs. Humans have four different Se-dependent GPX (Chu et al. 2004). These enzymes act by adding two electrons to reduce peroxides by forming selenoles (Se-OH). The substrate for the catalytic reaction is either H_2O_2 or an organic peroxide ROOH. Catalytic reactions can be described according to the following reactions:

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$$

$$2GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O$$

GPX competes with CAT for H_2O_2 as a substrate and is the major source of protection against low levels of oxidative stress. GR utilizes the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) to reduce one molar equivalent oxidized glutathione disulphide (GSSG) to two molar equivalents of sulfhydryl form of GSH:

$$GSSH + NADPH \xrightarrow{GR} 2GSH + NADP^{(+)}$$

GSH is not only the major multifunctional intracellular thiol antioxidant but also the key cellular redox buffer. GSH is highly abundant in the cytosol, nuclei and mitochondria. The oxidized form of GSH is GSSG. The antioxidant activity of all thiol compounds including GSH is their ability to accommodate a single electron originating from various types of radicals forming thiyl radicals ($GS^{\bullet}$) with much longer lifetime than $R^{\bullet}$ and reduced reactivity:

$$GSH + R^{\bullet} \rightarrow GS^{\bullet} + RH$$

GSSG is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of living systems. GSH reacts with $^{\bullet}OH$ and $^1O_2$ and regenerates important low-molecular-weight antioxidants. Decreased levels of GSH have been linked to a number of disease states of an organism including cancer; neurodegenerative, CVDs and pulmonary disease; HIV infection; acute pancreatitis; and others (Pastore et al. 2003).

12.3 Dietary Antioxidants

Vitamin C (ascorbic acid) is one of the most important and powerful antioxidants that acts in aqueous environments of the body. Vitamin C coacts with vitamin E to regenerate α-tocopherol from α-tocopherol radicals in membranes and lipoproteins (Chan 1993). A molecule of ascorbic acid has two ionisable hydroxyl groups. At physiological pH, the majority of vitamin C molecules are present as ascorbate anions ($AscH^-$) and only very small fraction as $AscH_2$ and Asc^{2-}. The antioxidant chemistry of vitamin C is thus the chemistry of $AscH^-$. Ascorbate anion is a donor antioxidant and reacts with radicals to form the ascorbate free radical ($Asc^{\bullet-}$) which is, due to its pK value (−0.86), not protonated and is present in the form of Asc^{2-} (Fig. 12.2). The ascorbate radical (Asc^{2-}) is considered as a poorly reactive terminal radical. The level of this radical is a good measure of the degree of oxidative stress in biological systems (Buettner 1993).

Vitamin E is a fat-soluble powerful antioxidant present in several forms. The most powerful form is α-tocopherol, which is the key membrane-bound antioxidant

(Sharma and Buettner 1993) and acts mainly against peroxidation of lipids. It has been proposed that ascorbic acid and α-tocopherol act together in a cyclic manner, in which ascorbic acid regenerates oxidized form of vitamin E (tocopherol radical, α-T-O•). The protective effect of vitamin E is substantiated by the suppressed formation of free radical formation as well as activation of endonucleases. The protective effect of the intake of vitamin E supplements (200 IU/day) against colorectal cancer has been reported by an epidemiological study. The protective effect was accounted for by the triggered apoptosis of cancer cells by inducing an efficient inhibitor of cell cycles ($p21^{wafi/cip1}$).

Flavonoids represent an important group of polyphenols with more than 4000 compounds divided into 13 classes. Their common structural element is the diphenylpropane (C6-C3-C6) moiety, which consists of two aromatic rings linked through three carbon atoms (Fig. 12.3) (Terao 2009). Recent interest in flavonoids has significantly increased mainly because of their antioxidant properties and their possible beneficial effects in human health (Terao 2009). These include the treatment and prevention of cancer, CVDs and other pathological disorders. Phenolic compounds (see Fig. 12.3) may act not only as antioxidants terminating ROS but also as effective chelators of transition metal ions that are capable of catalysing lipid peroxidation. Under unphysiological conditions, e.g. high concentrations of

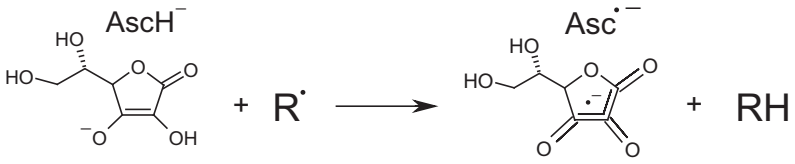

Fig. 12.2 Ascorbic acid and its reaction with radicals (R•)

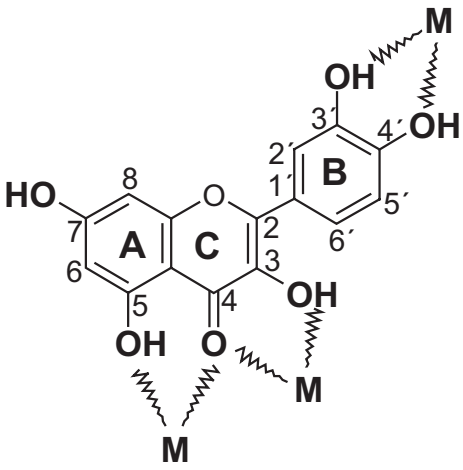

Fig. 12.3 Structure of a flavonoid quercetin. (*M* = coordinated metal ion)

flavonoids and presence of the transition metal ions, flavonoids may behave as pro-oxidants (Bast and Haenen 2013).

A regular intake of flavonoids has been associated with decreased incidence of gastrointestinal, lung and possibly breast cancers (Damianaki et al. 2000). A high intake of the flavonoid quercetin has been proposed to reduce the incidence of stroke. However, there have been studies presenting toxic effects of flavonoids as well as toxic flavonoid-drug interactions. The main symptoms were contact dermatitis, oestrogenic-related concerns and other problems. The toxic effect of flavonoids on human health has been attributed to pro-oxidant properties of flavonoids. We note that pro-oxidant effect of some antioxidants has previously been reported and was related to disturbed metabolism of redox metals as well as other unphysiological states of an organism. Thus the potential toxic effect of flavonoids under disturbed physiological conditions requires further detailed studies.

12.4 Oxidative Stress and Cardiovascular Disease

In the following, we will describe some evidence for an aetiology of CVDs due to free radical damage to cells pertinent to the healthy function of the cardiovascular system. The current most enlightening insights in the dysfunction of cardiovascular system include Ca^{2+} overload, oxidation of receptor sites, lipid peroxidation of membranes, perturbation of signalling systems and the mechanism of ischemic preconditioning. These will be described in more detail presently. Some of the substances believed to play a major role in oxidative stress in the cardiovascular system are (1) certain enzymes such as xanthine oxidoreductase (XOR), (2) NAD(P)H oxidase, (3) NOS, (4) cytochromes in mitochondria and (5) haemoglobin (Berry and Hare 2004; Hare and Stamler 2005). Biochemical pathways leading to $^{\bullet}O_2^-$ and $NO^{\bullet}$ production are given in Fig. 12.4.

ROS modify phospholipids and proteins, which lead to peroxidation and oxidation of thiol groups (Kuka et al. 2013; Molavi and Mehta 2004). Thus membrane permeability is changed with possible disruption of membrane bilayer. In addition protein function is disrupted or modified. One study (Kaneko et al. 1989) showed that oxygen free radical changes to sulfhydryl groups depressed sarcolemmal Ca^{2+} pump activities. Sarcolemma was incubated with H_2O_2 and Fe^{2+}. It was observed that ecto-ATPase activity was inhibited. The ATP-independent $Ca^{2+}$was also inhibited. After 1 min of incubation with $^{\bullet}O_2^-$, there was a 15% drop in the sarcolemmal ATP-dependent Ca^{2+} accumulation and Ca^{2+}-stimulated ATPase activities. These effects seem to correlate with an increase of malondialdehyde (MDA) in the sarcolemma. MDA is a mutagen for human cells (Niedernhofer et al. 2003) and thus will potentially further degrades cell function in this region of its occurrence. Xanthine plus xanthine oxidase or H_2O_2 caused a decrease in mitochondrial creatine kinase activity in the rat heart (Hayashi et al. 1998). They proposed that γ-glutamylcysteine ethyl ester was a suitable solution in counteracting this decrease. γ-Glutamylcysteine ethyl ester is a precursor of GSH, which is an important antioxidant but cannot be

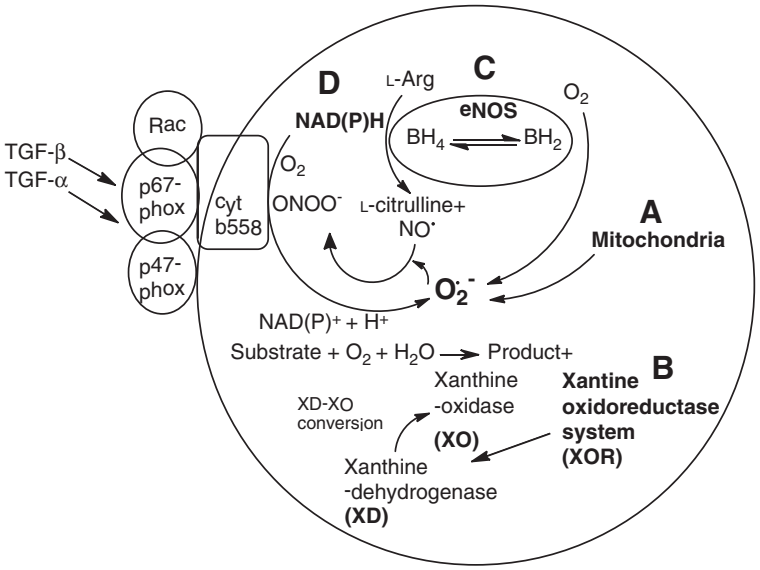

Fig. 12.4 Main pathways of ROS formation in cardiovascular system

administered directly due to degradation into constituent amino acids before entering cells (Jensen and Meister 1983).

The reduced efficiency of the Ca^{2+} pump mechanism leading to Ca^{2+} overload and resulting myocyte dysfunction has been well established in many other studies (Hua et al. 2015). ROS can have a direct effect on Ca^{2+}-handling proteins and/or membrane impairment by lipid peroxidation. This can reduce the efficiency of the pump mechanism. Stoyanovsky et al. (1997) report that $^{\bullet}O_2^-$, $^{\bullet}OH$ and $NO^{\bullet}$ promote sarcoplasmic reticular Ca^{2+} release by interaction with sulfhydryl groups of cardiac and skeletal ryanodine receptor. Other mechanisms of damage such as involving an increase in Na^+ and accumulation of long-chain fatty acids in cardiac membrane are also possible. ATP deficiency in the ischemic heart may also impair Ca^{2+} transport, and reperfusion may increase uptake of extracellular Ca^{2+} into the myocardium causing further overload. Intracellular Ca^{2+} overload appears to be associated with simulation of neointimal hyperplasia. This is believed to result in the development of atherosclerosis, vasoconstriction leading to development of hypertension, myocardial cell damage in ischemia-reperfusion and cardiac hypertrophy in heart failure.

ROS have been found to be involved in atherosclerosis. Animal models suggest large iron pools in atherosclerotic lesions. This may suggest a role of iron-catalysed formation of free radicals (Fenton reaction) in atherosclerosis (Yuan and Li 2003). Human endothelial cells show increased Ca^{2+} suggesting again Ca^{2+} overload induced oxidative stress. Uptake of oxidized low-density lipoprotein (LDLox) also seems to be important in the development of atherosclerosis (Podrez et al. 2000). It has been reported that oxidized lipoprotein and LDLox mediate enhanced $^{\bullet}O_2^-$ formation leading to apoptosis of cells in the umbilical vascular wall. LDLox-mediated

formation of ROS can cause plaque formation (Ruiz et al. 2005). Treatment with SOD and CAT can prevent these effects.

Oxidation of $NO^{\bullet}$ by $^{\bullet}O_2^-$ results in formation of $ONOO^-$, which initiates lipid peroxidation or lipoprotein oxidation. These are believed to be important events in development of atherosclerosis. Increased levels of $^{\bullet}O_2^-$ and H_2O_2 have been reported in hypertensive patients (Araujo and Wilcox 2014). $^{\bullet}O_2^-$ promotes cell proliferation while H_2O_2 induces apoptosis and activates protein kinase C. This suggests a role for protein kinase C. Hypertensive patients appear to have decreased levels of exogenous non-enzymatic antioxidants such as vitamin E and endogenous antioxidants such as GSH and SOD. Interaction of $^{\bullet}O_2^-$ and $NO^{\bullet}$ is believed to be involved in the process of hypertension (Li and Forstermann 2000). Elevated $^{\bullet}O_2^-$ levels linked with suppressed formation of $NO^{\bullet}$ from aortic rings were observed in a renal hypertension rat model. Vascular endothelial cells are known to generate $NO^{\bullet}$, so suppressed levels of this species are suggestive of endothelial dysfunction.

Angiotensin II (AngII) is a multifunctional hormone (Zucker et al. 2015). It influences cell growth, apoptosis, migration, inflammation and fibrosis (Romero and Reckelhoff 1999; Sowers 2002). It regulates blood pressure and fluid homeostasis. Evidence suggests that ROS production is tightly linked with excessive AngII-induced action. For example, AngII increases ROS by vascular smooth muscle cells. AngII-induced hypertension has also been associated with increased vascular $^{\bullet}O_2^-$ production. Angiotensin-converting enzymes (ACE) produce AngII from AngI. Since overproduction of AngII leads to induced hypertension, a possible solution is to produce inhibitors, which inhibit ACEs. AngII will appear again when we discuss $NO^{\bullet}$ signalling.

Heart tissue is rich in cardiolipin (Santucci et al. 2014). This is a phospholipid acylated in four sites, mostly with linoleic acid. It has been found that cytochrome c is normally bound to inner mitochondrial membrane (Robinson 1993). Peroxidation of cardiolipin causes dissociation of cytochrome c, which then is released through the outer mitochondrial membrane into the cytosol. The exact mechanism of cytochrome release is not clear but may involve mitochondrial permeability transition (MPT) pore with swelling of matrix and rupture of outer membrane. This is consistent with growing evidence that ROS play some role in simulating cytochrome release from mitochondria. ROS may also cause MPT by oxidation of thiol groups on adenine nucleotide translocator believed to form part of MPT pore (Petrosillo et al. 2003). Cytochrome c can also be released by mechanisms involving an oligomeric form of β-cell lymphoma-2-associated protein, an apoptosis regulator, which does not involve MPT, with no swelling and rupture of the membrane (Jürgensmeier et al. 1998).

12.5 Oxidative Stress and Type 2 Diabetes

Type 2 diabetes mellitus is an increasingly common disease in the Western world leading to increased risk to CVDs and other complications. It was formerly known as non-insulin-dependent diabetes as patients are not 100% dependent on regular

injections of insulin as are type 1 diabetes patients. It is about nine times more common than type 1 diabetes. Type 2 diabetes is believed to be due to insufficient insulin production and insulin resistance which means that certain cells, such as in the muscles, liver and fat tissue, are unable to respond appropriately, even to normal levels of insulin. This causes abnormal carbohydrate, lipid and protein metabolism. Patients may vary in the degree of inadequate insulin production, and some individuals who have insulin resistance do not develop type 2 diabetes (Gustafson et al. 2015). In insulin resistance, liver cells release too much glucose into the blood. Over prolonged periods, high glucose levels are believed to cause further deterioration in the health of the patient.

Animal models used to study diabetes usually involve rats with diabetes induced by streptozotocin, which is toxic to the insulin-producing pancreatic β-cells (Rossini et al. 1977).

It is well known that type 2 diabetes involves the dysfunction of pancreatic β-cells. Many studies have suggested that this dysfunction is a result of prolonged exposure to high glucose and elevated free fatty acid levels (Evans et al. 2003). This suggests that a high-sugar and high-fat diet is partly responsible and may perhaps be avoidable by a healthy diet.

Changes in genetic expression have also been reported to occur as a mechanism in β-cell toxicity. Robertson and colleagues suggest involvement of pancreas duodenum homobox-1 (PDX-1) and insulin gene expression (Robertson et al. 2003). Chronic exposure of HIT-T15 cells to supra-physiological concentrations of glucose over several months causes gradual loss of insulin gene expression (Robertson et al. 2003). It is thought that the mechanism involves loss of mRNA and protein levels of the PDX-1 gene. This may involve N-terminal kinase (JNK) pathway interfering with PDX-1 gene expression and is the subject of further investigation.

The role of oxidation in the formation of type 2 diabetes is particularly convincing when it was discovered that pancreatic β-cells, compared with other cell types, are low in antioxidant enzymes such as SOD, CAT and GPX (Grankvist et al. 1981). This makes them likely to be particularly sensitive to oxidative stress compared with many other types of cells. It was shown that oxidative stress by short exposure of β-cell to H_2O_2 increased production of cyclin-dependent kinase (CDK) inhibitor $p21^{WAF/CIP1/Sdi1}$ and decreased insulin mRNA, cytosolic ATP and calcium flux in cytosol and mitochondria (Kaneto et al. 1999).

The insulin receptor is composed of two extra cellular α-subunits and two transmembrane β-subunits linked by –S-S- bonds. The receptor processes an intrinsic tyrosine kinase activity. When activated by attachment of insulin to α-subunit, the receptor is phosphorylated on the tyrosine residue on the β-subunit (Lawlor and Alessi 2001). The activated receptor can then phosphorylate the insulin receptor substrate (IRS) proteins and other substrates. Phosphorylation leads to activation of different signalling pathways. The activation of phosphatidylinositol 3-kinase (PI 3-kinase) is involved in the metabolic functions of insulin. IRS1 and IRS2 are the most important substrates in insulin signalling. IRS1 can be phosphorylated on serine residues. This phosphorylation of IRS1 has a dual role either enhancing or

terminating effect of insulin. An imbalance between positive IRS1 tyrosine phosphorylation and negative IRS1 serine phosphorylation is strongly stimulated by "diabetogenetic" factors including free fatty acids, tumour necrosis factor alpha (TNFα) and oxidative stress. Insulin-activated protein kinase B (PKB) propagates insulin signalling and promotes phosphorylation of IRS1 on serine residue. This creates positive feedback for insulin action.

Insulin resistance-inducing agents such as angiotensin II, cytokines, free fatty acids, endothelin-1, cellular ROS and hyperinsulinemia can activate several serine/threonine kinases and also phosphorylate IRS1 (Vicent et al. 2003). These agents, which negatively regulate IRS1 by phosphorylation, also operate via other mechanisms such as suppression of the expression of cytokine signalling proteins (SOCS), IRS degradation and O-linked glycosylation. Clearly an understanding of the mechanisms of IRS1 inhibition and the identification of kinases involved may help in designing therapies to prevent insulin resistance.

Normally $^{\bullet}O_2^-$ is predominately produced at the sites in the mitochondrial membrane known as complex I and the ubiquinone-complex III interface. This is where long-lived intermediates allow reaction time for electrons with molecular dioxygen (Kwong and Sohal 1998). In diabetes, however, the sites are changed so that complex II becomes the main site of $^{\bullet}O_2^-$ production (Nishikawa et al. 2000). NADPH oxidase enzymes are a family of transmembrane proteins, which transport electrons across the membrane. It converts NADPH to $NADP^+$. NADPH is an electron donor and oxygen outside the cell is the electron acceptor. The result produces $^{\bullet}O_2^-$. Clearly there is potential here to be a source of ROS. Evidence supports the assertion that NADPH oxidase is a major source of glucose-induced ROS production in vascular and kidney cells suggesting NADPH as a mediator of diabetes complications (Li and Shah 2003). The cytosolic component of activated NADPH known as $p47^{phox}$ can be blocked with AngII type 1 receptor antagonists. NADPH oxidase production of ROS in diabetes can be suppressed by a variety of PKC inhibitors.

Hyperglycaemia-induced oxidative stress also occurs in nonnucleated cells (erythrocytes) lacking mitochondria and NADPH oxidase. Therefore there must be other sources of ROS production in these cases. One possible explanation is glucose autoxidation (Robertson et al. 2003). Glucose and its metabolites react with H_2O_2 in presence of iron and copper ions to form $^{\bullet}OH$. In vivo experiments have been attempted. Semchyshyn and his group (Semchyshyn et al. 2014) used intact *Saccharomyces cerevisiae* cells as in vivo model to investigate autoxidation of both glucose and fructose comparing results with in vitro experiments. They showed that in vitro fructose was more reactive than glucose and produced higher levels of autoxidation and glycation products. However, no substantive differences were observed for the effect of glucose and fructose on the intracellular level of glycoxidation products, when intact yeast cells were exposed to the high concentration of hexoses. Increases in the activities of SOD, CAT, glyoxalases and GR in both glucose- and fructose-stressed yeasts were found suggesting a reaction against the presence of oxidative/carbonyl stress. Glucose-6-phosphate dehydrogenase activity was found to decrease in yeast exposed to hexoses. Fructose was found to activate glyoxalases more than glucose.

$ONOO^-$ is highly reactive and is linked with diabetes and many other disease states (Zou et al. 2002). $ONOO^-$ reacts with the zinc cluster component of NOS so reducing the cells ability to produce $NO^\bullet$ in the places where it is desirable as a signalling molecule. Hyperglycaemia appears to be correlated with regulation of NOS expression and production of $ONOO^-$. Addition of PKC can suppress glucose-induced aortic expression of endothelial NOS (eNOS). This suggests that PKC activation is an important part of hyperglycaemia-induced NOS up-regulation, perhaps mediated by NF-κB (Hink et al. 2001).

It has been suggested that xanthine oxidase (XO) is a key source of ROS in diabetes mellitus (Butler 2000). It has been reported that the XO inhibitor allopurinol reduces oxidized lipid levels in plasma and improves blood flow in type 2 diabetes patients. Diabetes is associated with increased lipoxygenase expression, which results in eicosanoid formation. Eicosanoids are signalling molecules resulting from complex signalling pathways starting with arachidonic acid. Increased lipoxygenase expression implies these signalling pathways are overactive.

ROS and RNS deplete exogenous antioxidants as evidenced by low levels of vitamin E and vitamin C in plasma of diabetes patients. The depletion of such antioxidants leads to further oxidative damage and ROS/RNS accumulation. Hyperglycaemia and diabetes complications affect regulation of GPX expression, but the degree is somewhat variable and understanding in terms of cellular health is unclear (VanderJagt et al. 2001). The biomarkers of type 2 diabetes are MDA, GSH/GSSG ratio, S-glutathionylated proteins, F_2-isoprostanes, 3-nitrotyrosine (NO_2-Tyr) and advanced glycation end products (AGEs). The accumulation of MDA seems to play an important role in the consequences of oxidative stress in diabetes patients. One study (Wang et al. 2011) used a diabetic rat model, which showed that the administration of luteolin lowered some of the biomarkers such as MDA. The role of 4-hydroxy-nonenal (HNE) is less clear, but a few studies suggest there is accumulation of this substance in diabetes and activation of signalling pathways (Traverso et al. 2002). Isoprostanes are non-enzymatic products of arachidonic acid oxidation and are found at higher levels in plasma and urine of type 2 diabetes patients. Isoprostanes are popular as biomarkers because of specificity and sensitivity of detection. However, elevated isoprostane levels do not prove isoprostanes are a cause of the onset of diabetic complications but may be simply by-products.

Glucose is known to react directly with free amino groups in proteins and lipids to eventually form modified forms known as AGE (Ling et al. 2001). They appear to accumulate with age and also appear as biomarkers for diabetes. They can be identified in tissues by immunohistochemical techniques. They are found in many of the tissues in animal models for diabetes and type 2 diabetes patients. Certain tissues such as the liver, kidney and testis are more susceptible than others. Erythrocytes, especially the surface membranes, also contain large amounts of AGEs. AGEs can be broken down in the liver and kidneys and by macrophages. However, when there is excessive accumulation, there is tissue injury due to decreased solubility of tissue proteins. This is believed to give rise to oxidative stress in these tissues (Fu et al. 1998). It is believed that AGE formation contributes to diabetic complications and ageing in general.

The role of antioxidants in treatment of metabolic disease has been subject of various studies. In one study (Buchanan et al. 2002), treatment with troglitazone delayed or prevented onset of type 2 diabetes in high-risk Hispanic women. It is believed that this protective effect is associated with preserving pancreatic β-cell function reducing the secretory demands placed on β-cells by chronic insulin resistance. Unfortunately troglitazone has harmful effects when administered over longer periods and has been taken off the market. The low concentration of SOD, CAT and GPX in β-cells raises the evolutionary question of why this is the case. Attempts to treat diabetes with antioxidants appear to be beneficial. Antioxidant treatment appears to suppress β-cell apoptosis without causing cell proliferation. Furthermore the expression of PDX-1 was visible in nuclei of islet cells after antioxidant treatment (Kaneto et al. 1999).

12.6 Oxidative Stress and Cancer

A multistage mechanism in cancer development is characterized by multiple events occurring in a cell and can be described by three stages, initiation, promotion and progression. ROS interfere with all these three stages of carcinogenesis (Klaunig and Kamendulis 2004). Initiation stage involves DNA mutations that produce an altered cell followed by at least one round of DNA synthesis to repair the damage created during the initiation. DNA damage can be initiated by a variety of ROS, probably the most damaging being the $^{\bullet}OH$ formed via metal-catalysed Fenton reaction. An interesting direct correlation between size of tumours and the level of detected 8-oxodG adducts has been detected; thus, it may speculated that the level of oxidative adducts may be a key factor determining the transformation from benign to malignant tumours.

The typical feature of promotion stage is clonal expansion of initiated cells by the induction of cell proliferation and/or inhibition of programmed cell death (apoptosis) (Koff et al. 2015). This stage is a reversible process and requires tumour promotion stimulus. During this stage, a strong inhibition of antioxidant pool such as SOD and CAT has been observed. The ROS formation during this stage is the main line of ROS-induced promotion of tumour growth. The third and final stage of carcinogenesis is irreversible and involves molecular changes accompanying the transformation of cells from the preneoplastic to the neoplastic state. Genetic damage and breakage of chromosome integrity are typical features of this stage (see Fig. 12.5).

As already discussed above Mn-SOD catalyses the dismutation reaction. The role of Mn-SOD in cancer is far from clear (Behrend et al. 2003). It has been proposed that Mn-SOD may act as an indirect tumour suppressor protein. Overexpression of Mn-SOD has been related to increased levels of oxidative stress typical for cancer cells. Overexpressed Mn-SOD deplete $^{\bullet}O_2^-$ which in turn reduces ROS-mediated stimulation of cellular growth (Zhang et al. 2002). An association between cancer and various disorders of GSH-related enzyme functions has been reported (Pastore

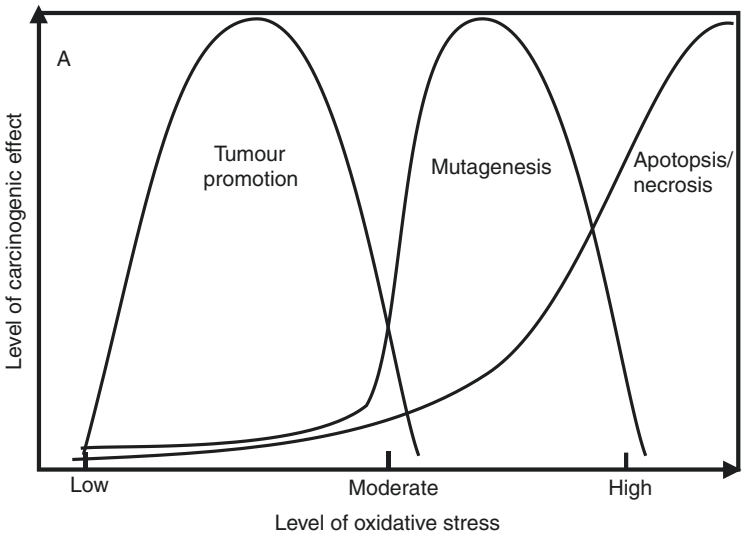

Fig. 12.5 The dependence of carcinogenic effect vs. level of oxidative stress at various stages of carcinogenic process

et al. 2003). The GSH/GSSG ratio estimated in the blood of patients with colon and breast cancers has been found to be significantly lowered compared to healthy subjects (Pastore et al. 2003). This has been clarified by an increased level of GSSG in advanced stages of cancer. This could be explained by stimulated formation of H_2O_2, which oxidizes GSH to GSSG in the red blood cells.

Low-molecular-weight antioxidants are involved in the conversion of ROS to less reactive species. However, antioxidant protection therapy in patients with advanced stages of cancer should be used very carefully, since the effects of antioxidants are strongly dependent on the stage of disease (Valko et al. 2004; Dreher and Junod 1996). Apoptosis is known to be stimulated by elevated levels of free radicals; therefore, depletion of free radicals due to the excessive administration of antioxidants might in fact stimulate survival of damaged cells and proliferation into neoplastic state and thus rather promote process of carcinogenesis than interrupt it. Antioxidant therapy during the progression stage may stimulate growth of tumours via enhanced survival of tumour cells. Pro-oxidant character of some antioxidants is also of significant importance (Mortensen et al. 2001; Valko et al. 2004).

12.7 Oxidative Stress and Alzheimer's Disease

Toxicity and oxidative stress linked with Aβ is substantiated by the disturbed metabolism of redox active metals such as iron and copper and non-redox metal zinc (Cuajungco et al. 2005). An important step in the confirmation of a significant role

of disturbed metabolism of redox active metals in Alzheimer's tissue has been made by applying three advanced physical techniques. A combination of scanning transmission ion microscopy, Rutherford backscattering spectrometry and particle-induced X-ray emission in conjunction with a high-energy (MeV) proton microprobe revealed increased concentration of metals in the amyloid plaques compared with the surrounding tissues (Rajendran et al. 2009). The level of iron in amyloid plaques was found to be doubled; copper and zinc were estimated to be nearly triple of that surrounding tissue. These data document the catalytic role of transition metal ions in the formation of oxidized species which in turn contribute to the occurrence of oxidative stress in brain tissues. Copper binds to Aβ via histidine (His13, His14, His6) and tyrosine 10 (Tyr10) amino acid residues. In addition to Cu(II), Aβ also binds Zn(II) and Fe(III). Under in vitro conditions, Zn(II) precipitates Aβ. Cu(II) interaction with Aβ promotes its neurotoxicity which has been substantiated by the reduction of Cu(II) to Cu(I) and the formation of H_2O_2 (Cuajungco et al. 2000). The copper complex with Aβ has a highly positive reduction potential, characteristic of strongly reducing cupro-proteins (Huang et al. 1999).

The role of antioxidants in AD has been subject of various studies. It has been reported that Aβ stimulates copper-mediated oxidation of ascorbate (Dikalov et al. 2004). Based on this study, it was concluded that toxic Aβ peptides stimulate copper-mediated oxidation of ascorbate ($AscH^-$) and generation of $^{\bullet}OH$ which in turn may be involved in the pathogenesis of AD. The mechanism can be described as follows:

$$A\beta\text{-}Cu(II) + AscH^- \leftrightarrow A\beta\text{-}Cu(I) + Asc^{\bullet -} + H^+$$
$$A\beta\text{-}Cu(II) + Asc^{\bullet -} \leftrightarrow A\beta\text{-}Cu(I) + Asc$$
$$A\beta\text{-}Cu(I) + H_2O_2 \rightarrow A\beta\text{-}Cu(II) + {}^{\bullet}OH + OH^- \text{ (Fenton)}$$
$$A\beta\text{-}Cu(I) + O_2 \leftrightarrow A\beta\text{-}Cu(II) + {}^{\bullet}O_2^-$$

Cupric ions in the presence of H_2O_2 may catalyse ROS oxidation of the peptide via the Fenton reaction. Using electron spin resonance spectroscopy, it has been shown that the N-terminal residues of His13, His14, His6 and Tyr10 are involved in the complexation of Cu with Aβ. It has been proposed that N-terminally complexed Cu(II) is reduced by electrons originating from the C-terminal methionine residues according to a reaction (Pogocki 2003) forming the radical of Met35 ($MetS^{\bullet +}$) and reducing cupric ions to cuprous species:

$$MetS + A\beta\text{-}Cu(II) \leftrightarrow MetS^{\bullet +} + A\beta\text{-}Cu(I)$$

While from a thermodynamic point of view reduction potentials of the Cu(II)/Cu(I) and Met/$MetS^{\bullet +}$ couples are rather unfavourable, electron transfer between MetS and Aβ-Cu(II) may be accelerated by the subsequent exergonic reaction of deprotonation of $MetS^{\bullet +}$, leaving behind the 4-methylbenzyl radical, thus making the reaction viable in vivo (Pogocki 2003). The sulphide radical $MetS^{\bullet +}$ may also undergo very fast reactions with $^{\bullet}O_2^-$. This reaction is substantiated by the formation of Met-sulphoxide (MetO) which has been isolated from AD senile plagues:

$$MetS^{\bullet +} + {}^{\bullet}O_2^- \xrightarrow{\text{Met}} 2MetO$$

The amino acid methionin-35 is strongly related to the pathogenesis of AD, since this amino acid is susceptible to oxidation under vivo conditions. It has been proposed that methionin-35 oxidation to Met-sulphoxide reduced toxic and pro-apoptotic effects of the Aβ protein fragment on isolated mitochondria (Pogocki 2003). As a consequence of broken metabolism of metals, a variety of oxidative products have been detected in AD brains. They include HO-1,8-hydroxy-guanine and oxidative modification of proteins, lipids and nucleic acids. Mainly lipid peroxidations are increased in the AD brain as compared with controls. In addition, the role of metals is linked with lipid peroxidation. Markers of lipid peroxidation detected in AD brains include HNE, 4-oxo-*trans*-2-nonenal (4-ONE), acrolein and 4-oxo-*trans*-2-hexenal, all of which are well-recognized neurotoxic agents.

Vitamin C levels of plasma in patients suffering AD have been found to be decreased as compared to control patients (Sultana et al. 2013). Levels of ascorbate in cerebrospinal fluids were also found to be decreased in AD patients compared to control subjects. This may suggest suppressed reduction of α-tocopherol radical of vitamin E back to α-tocopherol. The synergistic effect of vitamins C and E was studied in AD patients (Li et al. 2012). The combination of both vitamins E and C led to the increase of vitamins E and C in plasma and cerebrospinal fluids, making them thus less prone to in vitro oxidation. However, supplementation of vitamin E alone to AD patients did not show protection against in vitro oxidation. This study confirms the importance of synergism between vitamins E and C in patients with AD.

Flavonoids have been shown to be beneficial in patients with AD (Solanki et al. 2015). Neurotoxicity induced by Aβ, whose deposition in the brain accompanies neuronal loss in AD, was shown to be attenuated in the presence of epigallocatechin gallate. Epigallocatechin gallate is currently under investigation for its role as a chemoprotective agent. In addition, catechins are of interest due to their ability to directly scavenge ROS and RNS and exert indirect antioxidant effects via activation of transcription factors and antioxidant enzymes, modulating thus the cellular redox state.

12.8 Conclusions

Oxidative stress and nitrosative stress are mediators of damage to all cellular components which in turn may lead to the development of various diseases. ROS-induced oxidative stress in cardiac and vascular myocytes has been linked with cardiovascular tissue injury. The most profound signs of oxidative stress have been noted in ischemic heart disease, atherosclerosis, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure. Formation of ROS modifies phospholipids and proteins leading to peroxidation and oxidation of protein thiol groups. A critical factor in CVDs is the role of intracellular Ca^{2+} overload which can be induced by direct effect of ROS on Ca^{2+}-handling proteins or indirectly, by inducing membrane lipid peroxidation. In addition, other mechanisms involving an increase in the concentration of Na^{+} and accumulation of long-chain fatty acids in cardiac membranes should be considered.

Oxidative stress has been considered to be one of the major causes of the hyperglycaemia-induced diabetes mellitus. Hyperglycaemia stimulates generation of ROS from a variety of sources. These involve oxidative phosphorylation, glucose autoxidation, NAD(P)H oxidase, lipoxygenase, cytochrome P450 monooxygenases and NOS. The role of antioxidants in treatment of metabolic disease has been subject of various studies. The low concentration of key antioxidant enzymes in β-cells was observed. In line with this, attempts to treat diabetes with antioxidants appear to be beneficial. Antioxidant treatment appears to suppress β-cell apoptosis without causing cell proliferation.

The participation of ROS at various stages of the development of cancer is evident; many issues regarding the exact role of ROS and RNS in the aetiology of multifactorial diseases such as cancer are yet to be discovered. Of key importance is to characterize qualitatively and quantitatively which product of oxidative damage would be a suitable biomarker for cancer incidence. The main signs of metal-induced oxidative damage in AD patients involve oxidative modification of proteins, lipids and nucleic acids, mainly through lipid peroxidations. In addition, the role of metals is linked with lipid peroxidation. Markers of lipid peroxidation detected in AD brains include HNE, 4-ONE, acrolein and 4-oxo-*trans*-2-hexenal, all of which are well-recognized neurotoxic agents.

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Chapter 13
Protective Effects of Dietary Polyphenols in Human Diseases and Mechanisms of Action

Tao Shen, Xiu-Zhen Han, Xiao-Ning Wang, Pei-Hong Fan, Dong-Mei Ren, and Hong-Xiang Lou

Abstract More than 8000 polyphenols have been identified from plants, and several hundreds of dietary polyphenols were characterized in foods, such as fruits, vegetables, grains, dry legumes, chocolate, and plant-derived beverages such as fruit juices, tea, coffee, and red wine. Polyphenols have potent antioxidant capacity because of the presence of hydroxyl groups in their structures and thus they can directly scavenge reactive oxygen species (ROS). Based on their structural characteristics, dietary polyphenols are divided into phenolic acids, flavonoids, stilbenes, tannins, and miscellaneous dietary polyphenols. There is evidence that consumption of foods and beverages rich in polyphenols plays a role in the prevention of inflammation-related diseases, cancer, cardiovascular diseases, neurodegenerative diseases, diabetes, obesity, and osteoporosis. Modulation of multiple cell signaling pathways, including transcription factor NF-E2-related factor 2, nuclear factor-κB, mitogen-activated protein kinases, cytokines, cyclooxygenases, lipoxygenases, apoptosis-related proteins, and cell cycle proteins could explain the antioxidant actions of dietary polyphenols. Their diverse pharmacological functions suggest that dietary polyphenols are compounds with good preventive potential in human diseases. In this chapter, we illustrate the predominant members of dietary polyphenols and their protective effects and mechanisms of action against ROS-induced oxidative stress and associated noncommunicable diseases.

Keywords Dietary polyphenols • Flavonoids • Oxidative stress • Cancer • Cardioprotection • Neurodegeneration • Diabetes • Inflammation

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13.1 Introduction

Besides the traditional nutrients, many secondary metabolites of plants have the capacity to alter cellular functions and thus can have positive or negative roles for human health. These bioactive secondary metabolites are known as phytochemicals, phytonutrients, and nontraditional nutrients (Beecher 2003). Polyphenols are the largest group of nontraditional nutrients that have health benefits. It is estimated that human dietary intake of polyphenols is about 1 g/day, much higher than other phytochemicals or dietary antioxidants. It is approximately ten times higher than the consumption of vitamin C and about 100 times higher than the intake of vitamin E and carotenoids (Scalbert and Williamson 2000; Scalbert et al. 2005a, b). The scientific interest in the role of polyphenols for maintaining human health approximately started in the 1990s and significant progress has been made since then (Kroon and Williamson 2005).

Polyphenols are characterized by the presence of one or several phenolic groups in their structure and enjoy widespread distribution in the plant kingdom. Fruits, vegetables, chocolate, cereals, and plant-derived beverages such as fruit juices, tea, coffee, and red wine are the main dietary source of polyphenols. Polyphenols show highly diverse structures, of which, phenolic acids, flavonoids, stilbenes, and tannins are the main structural types of dietary polyphenols. Evidence from a number of studies has established correlations between the intake of polyphenols and reduced incidence of some human diseases and verified their roles in the prevention of inflammation-related pathologies, cancer, cardiovascular diseases, neurodegenerative disorders, diabetes, obesity, and osteoporosis (Scalbert et al. 2005a, b; Pandey and Rizvi 2009). Some commercial products with polyphenols as dominant constituents have been sold in the market and used for the prevention of some human noncommunicable diseases (NCDs) (Table 13.1).

It is well known that polyphenols have potent antioxidant capacity because of the presence of hydroxyl groups in their structures and thus they can directly scavenge reactive oxygen species (ROS). However, their antioxidant capacity appears to be only a part of the reason for its benefits in human health, as other mechanisms have been proposed for the actions of dietary polyphenols, including the regulation of transcription factor NF-E2-related factor 2 (Nrf2), nuclear factor-κB (NF-κB), activator protein-1 (AP-1), mitogen-activated protein kinases (MAPKs), cytokines, cyclooxygenases (COXs), lipoxygenases (LOXs), apoptosis-related proteins (Bcl-2, caspases), and cyclin-dependent kinases (CDKs) (Upadhyay and Dixit 2015). These findings provide new insights on the preventive effects of dietary polyphenols against NCDs. Experimental and epidemiological studies have added much to our knowledge of the protective effects of dietary polyphenols in NCDs and their mechanisms of action (Manach et al. 2004; Scalbert et al. 2005a, b; Fresco et al. 2006; Han et al. 2007; Pandey and Rizvi 2009; Tsao 2010; Chuang and McIntosh 2011; Del Rio et al. 2013; Ali et al. 2014; Upadhyay and Dixit 2015). In this chapter, we illustrate the main types and typical constituents of dietary polyphenols, summarize their preventive and therapeutic functions in several NCDs, and also discuss their mechanisms of action.

Table 13.1 Some commercially available polyphenol products and prevention of human diseases

Name	Components	Uses
Green tea extract	EGCG, (−)-epicatechin. (−)-epicatechin-3-gallate, (−)-epigallocatechin	Scavenging reactive oxygen species, promoting weight loss, inducing enzymatic phase II detoxification, enhancing immune function, protecting brain tissues, lowering blood cholesterol, LDL, and triglyceride levels
Grape seed extract	Oligomeric proanthocyanidins, resveratrol	Eliminating free radicals, preventing cancer, cardiovascular diseases (e.g., atherosclerosis, high blood pressure, high cholesterol, and poor circulation), and complications related to diabetes
Resveratrol	Resveratrol	Reducing the risk of cancer, heart diseases, diabetes, and Alzheimer's disease; preventing the side effects of obesity
Apple wise polyphenol Extract	Phloridzin, chlorogenic acid	Reducing triglyceride absorption from the intestine; inhibiting lipid peroxidation and inflammatory responses; regulating blood glucose levels
Ginkgo biboba extract	Flavonoids, ginkgolides	Improving memory, dementia, multiple sclerosis, sexual dysfunction, premenstrual syndrome, dizziness, and vertigo
Soy isoflavone	Daidzein, genistein	Preventing osteoporosis and breast cancer, improving menopausal symptoms, and premenstrual syndrome
Turmeric complex	Curcumin	Preventing cancer, Alzheimer's disease, cardiovascular disease, and inflammatory diseases

13.2 Classification of Dietary Polyphenols

More than 8000 polyphenols have been identified from plants, and several hundreds of dietary polyphenols were characterized in edible foods, such as fruits, vegetables, and grains (Manach et al. 2004; Han et al. 2007). Dietary polyphenols can be classified into groups according to their source of origin such as tea polyphenols, grape polyphenols and apple polyphenols, or their chemical structures. In this review, we use chemical structures as the criterion for classification of dietary polyphenols. These molecules were grouped into phenolic acids, flavonoids, stilbenes, tannins, and miscellaneous dietary polyphenols. Of these, flavonoids and phenolic acids, approximately accounting for 60% and 30% of dietary polyphenols, respectively, are the two principal types.

13.2.1 Phenolic Acids

Phenolic acids are a group of low-molecular-weight substances containing a polyhydroxy phenolic ring and organic carboxylic acid functionality in their structures and are widely distributed in plant kingdom (Robbins 2003). Phenolic acids in the diet can be divided into two subgroups: hydroxybenzoic acid derivatives (C6-C1 skeleton) and hydroxycinnamic acid derivatives (C6-C3 skeleton). Despite their

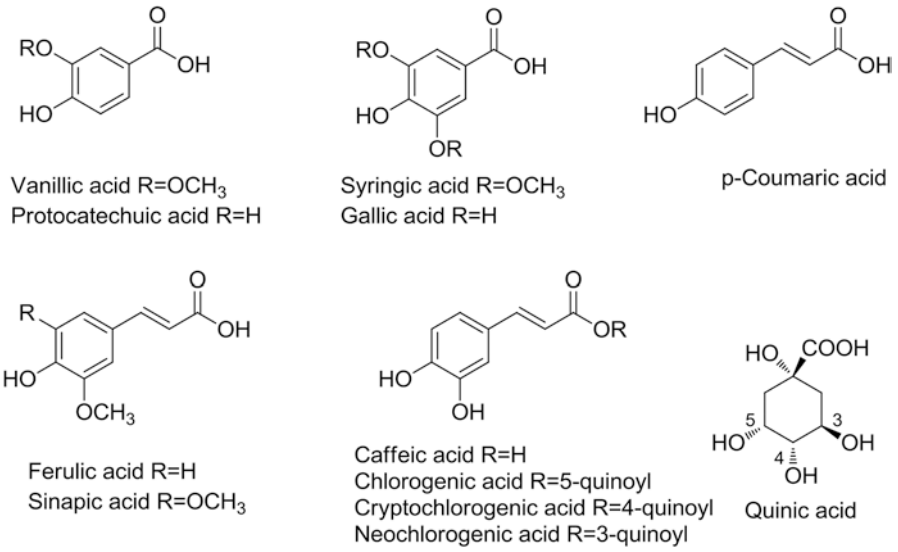

Fig. 13.1 Structures of typical dietary phenolic acids

simple basic skeletons, structural variety of phenolic acids is created by variation of the numbers and positions of the hydroxyl groups on the aromatic ring, along with the varied substitutions on the carboxyl group. The representatives of phenolic acids encompass vanillic acid, protocatechuic acid, gallic acid, and syringic acid as hydroxybenzoic acid derivatives and p-coumaric acid, caffeic acid, chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid, ferulic acid, and sinapic acid as hydroxycinnamic acid derivatives (Fig. 13.1).

13.2.2 Flavonoids

Flavonoids are commonly characterized by the linkage of two aromatic rings through three carbons (C6-C3-C6 skeleton) and represent the most widely distributed plant and/or dietary polyphenols (Beecher 2003). Their parent nucleus is shown in Fig. 13.2. Based on the connecting position of an aromatic ring (ring B) to the heterocyclic ring (ring C), the oxidation state and functional groups of the heterocyclic ring (ring C), flavonoids can be classified into several subgroups: flavones, flavonols, flavanones, flavanonols, isoflavones, isoflavanones, chalcones, dihydrochalcones, flavan-3-ols, and anthocyanins. The basic structures of the main flavonoids are shown in Fig. 13.2. Despite some evidence for structural variations that have enriched the subclass of flavonoids, herein we specifically focus on the subgroups that are related to dietary polyphenols. The flavonoid nucleus can be substituted by various groups to give a wide variety of flavonoid derivatives that are further enlarged by their structural diversity via chemical reactions of oxidation and cyclization. Flavonoid aglycones, glycosides, biflavones, prenylated flavanones,

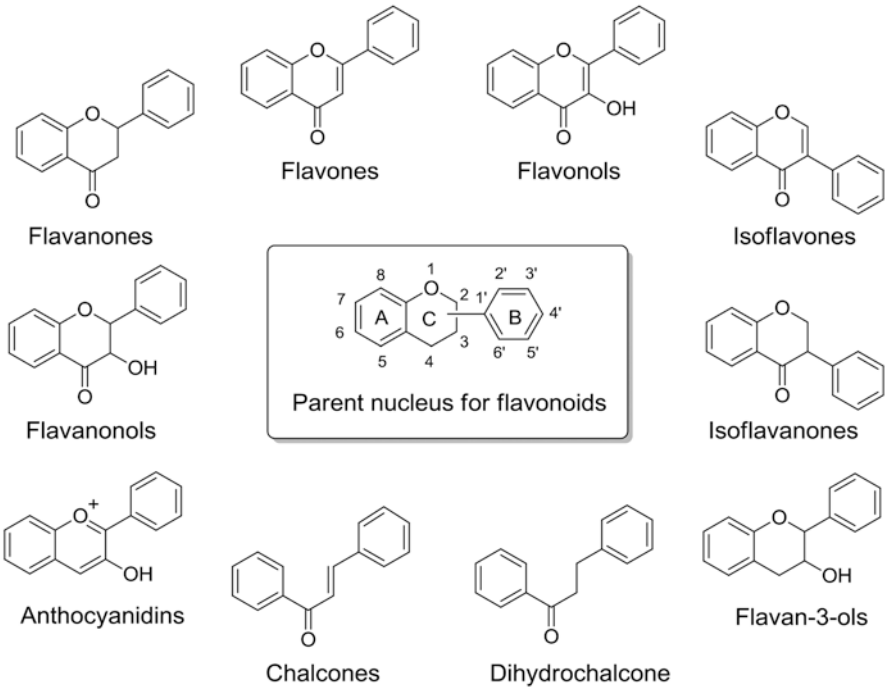

Fig. 13.2 Basic structures for different types of flavonoids

furanoflavanones, pyranoflavanones, and benzylated flavanones are the common forms of flavonoids in the plant kingdom. Accordingly, structural diversity of flavonoids gives rise to their biodiversity.

Flavones, flavonols, flavanones, and flavanonols with the aromatic ring B attached to the C2 position of heterocyclic ring C in their structures are the most widespread types of flavonoids. Apigenin and luteolin attributed to flavones; kaempferol, quercetin, myricetin, and isorhamnetin to flavonols; and eriodictyol, naringenin, and hesperetin to flavanones are the 26 prominent dietary flavonoids in the United States Department of Agriculture (USDA) flavonoid databases and are widely distributed in fruits, vegetables, nuts, seeds, spices, and herbs (Bhagwat et al. 2011). A flavanonol taxifolin has been found in some citrus fruits (Kawaii et al. 1999). The structures for these prominent dietary flavonoids and the above four flavonoid subtypes are shown in Fig. 13.3.

In contrast to the four subgroups shown above, isoflavones and isoflavanones bear a C3 replacement of ring B in the heterocyclic ring C. The representatives of isoflavones include daidzein, genistein, glycitein, biochanin A, formononentin, and their glycosides (Fig. 13.4), and mainly occur in soybeans, soy food, and legumes, and are typically exemplified by tofu (Setchell and Cassidy 1999). These constituents are known as phytoestrogens because of their capacity of exerting estrogen-like physiologic effects in the human body. Another atypical group of flavonoids are chalcones and dihydrochalcones, which are characterized by the presence of a

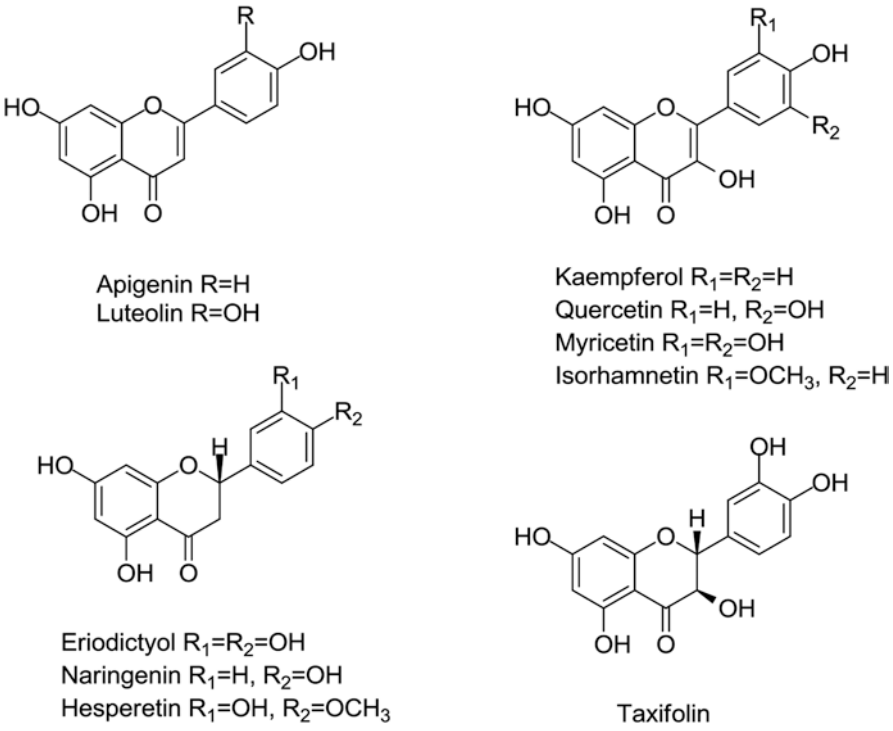

Fig. 13.3 Structures of typical dietary flavones, flavonols, flavanones, and flavanonols

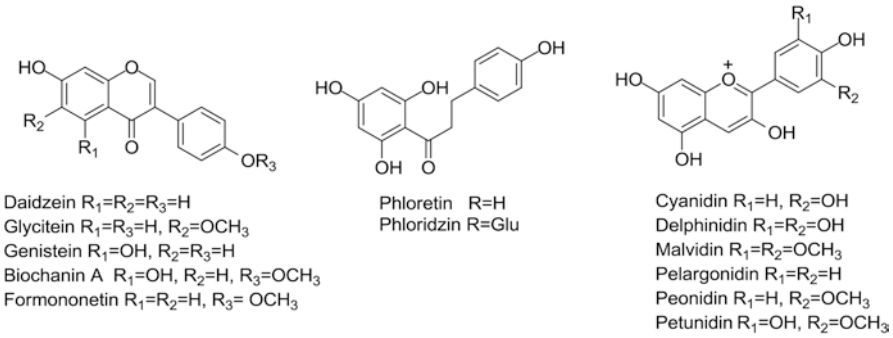

Fig. 13.4 Structures of typical dietary isoflavones, dihydrochalcones, and anthocyanins

cleaved heterocyclic ring C. Phloridzin and its aglycone phloretin belonging to dihydrochalcones are found in the fruit of *Malus* plants, such as apples (Fig. 13.4) (Gosch et al. 2010). They have attracted much attention due to their potential hypolipidemic and hypoglycemic functions.

Anthocyanidins are an important group of water-soluble pigments in plants and confer the bright red, blue, purple, and intermediate hues of flowers, fruits, and vegetables (Clifford 2000). Their hue and chemical structure vary with changes of pH

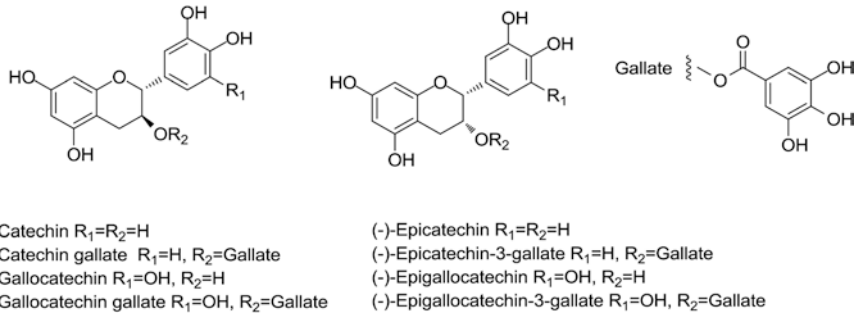

Fig. 13.5 Structures of typical dietary flavan-3-ols

and the presence of copigments. Different types of flavonoids, anthocyanidins carry a positive charge under acidic conditions. The most common anthocyanidin skeletons are cyanidin, delphinidin, pelargonidin, malvidin, peonidin, and petunidin (Fig. 13.4), which belong to the 26 prominent dietary flavonoids recorded in the USDA flavonoid databases (Bhagwat et al. 2011). Introduction of various sugar moieties, such as glucose, galactose, rhamnose, and arabinose, at different positions of anthocyanidin skeletons generates several hundreds of different plant anthocyanins. Anthocyanins are the most consumed dietary flavonoids and their daily intake in the USA is about ninefold higher than that of other dietary flavonoids (Wang and Stoner 2008).

Flavan-3-ols are commonly referred to as catechins, which possess two aromatic rings (rings A and B) and a dihydropyran heterocycle (ring C) with a hydroxyl group at C3 position (Fig. 13.5). Removal of the double bond at ring C and the two chiral centers (C2 and C3) in the structures of catechins enables them to exist as stereoisomers. Taking catechin, for instance, the isomer with *trans* configuration of C2 and C3 is defined as a catechin, while the *cis* configuration is epicatechin. Each of these two configurations has two stereoisomers that are named as (+)-catechin, (−)-catechin, (+)-epicatechin, and (−)-epicatechin. The important representatives of this group include (+)-catechin, (−)-epicatechin. (−)-epicatechin-3-gallate, (−)-epigallocatechin, (−)-epigallocatechin-3-gallate (EGCG), and (+)-gallocatechin, which are prominent dietary flavonoids in the USDA flavonoid databases (Bhagwat et al. 2011). Catechins enjoy wide distribution in the human diet and are particularly present in the skins of apples, grapes, and blueberries, as well as in cacao bean and tea (Crespy and Williamson 2004; Lamuela-Raventós et al. 2005).

13.2.3 Tannins

Tannins are a group of highly hydroxylated plant metabolites initially defined as water-soluble phenolic compounds having molecular weights between 500 and 3000 Da (Haslam 1996). Since then, tannins with a molecular mass greater than 30,000 Da were discovered in carob pods (Wursch et al. 1984). Besides undergoing common phenolic reactions, they can form insoluble complexes with carbohydrates

and protein and precipitate alkaloids and gelatin. Therefore, tannin-rich foods, such as hawthorn and pomegranate are astringent due to the precipitation of salivary proteins. Tannins can be classified into two subgroups: hydrolysable tannins and condensed tannins. Hydrolysable tannins exist in the form of multiple esters produced by esterification of gallic acid and its dimmer hexahydroxydiphenic acid with a core polyol, which is commonly glucose. The galloyl group is further esterified to form more complex hydrolysable tannins. As the name implies, hydrolysable tannins are hydrolyzed under acidic and basic conditions to yield polyol and aromatic acids. Tellimagrandin II and rugosin D are two representatives of hydrolysable tannins found in meadowsweet (Fig. 13.6) (Haslam 1996).

Condensed tannins are formed by the polymerization of the fundamental flavan-3-ol nucleus, such as catechin, epicatechin, afzelechin, and epiafzelechin. They are generally known as proanthocyanidins because depolymerization of these molecules under oxidative conditions yields anthocyanidins. Proanthocyanidins exclusively polymerized by catechin and/or epicatechin units are designated as procyanidins, which are the predominant type of proanthocyanidins and widely distributed in fruits, such as grapes, peaches, apples, and pears, as well as wine and tea (Rasmussen et al. 2005). While proanthocyanidins consist of (epi)afzelechin or (epi) gallocatechin, they are termed propelargonidins and prodelphinidins, respectively. Proanthocyanidins exist as polymers with degrees of polymerization ranging from 2 to greater than 50 (Fig. 13.7) and display diverse biological activities, especially antioxidant capacity. Proanthocyanidins are the main bioactive polyphenols in red wine thought to reduce the risk of coronary artery disease (Corder et al. 2006). A series of unique tannins derived from coupling of (−)-epigallocatechin or EGCG,

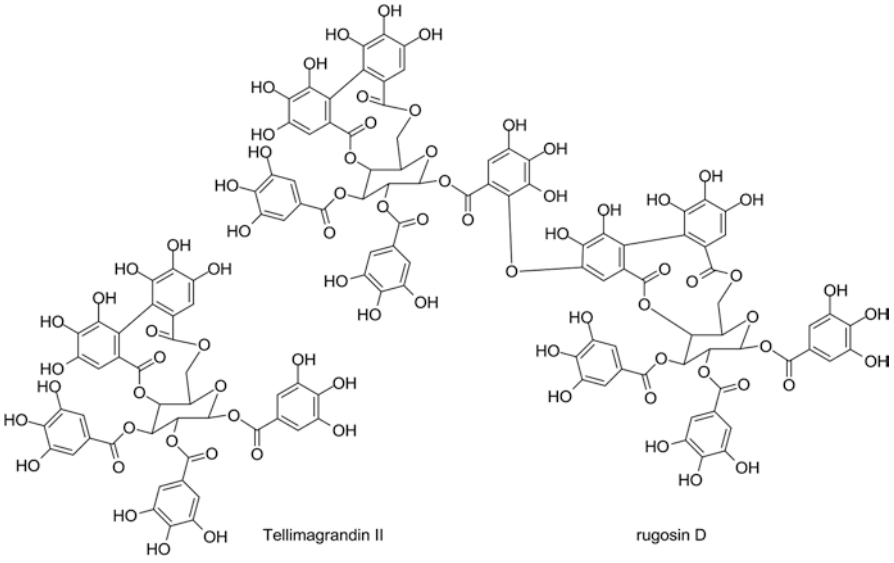

Fig. 13.6 Structures of typical hydrolysable tannins in the human diet

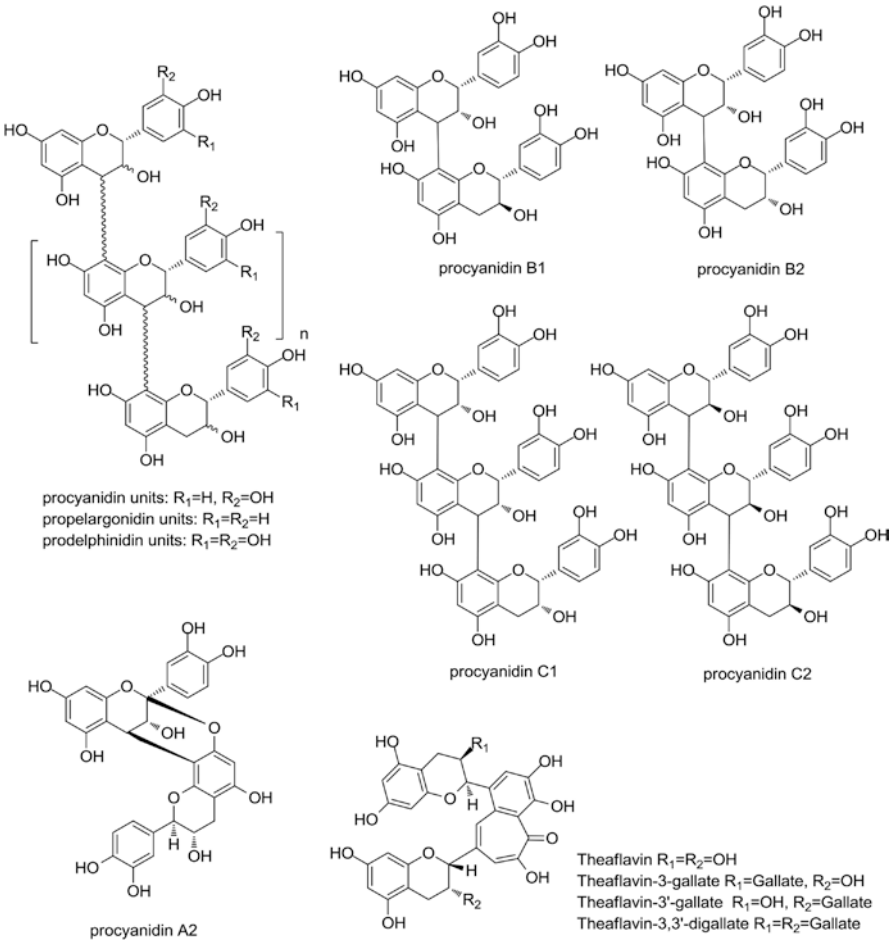

Fig. 13.7 Structures of typical dietary condensed tannins

named theaflavins, are important phenolic constituents in the brewed tea (Bhagwat et al. 2011). Representatives of procyanidins, including dimmers B1, B2, A2 and trimmers C1 and C2, as well as theaflavins are shown in Fig. 13.7.

13.2.4 Stilbenes

Stilbenes are structurally characterized by the presence of a 1,2-diphenylethylene nucleus and have attracted a lot of interest due to their potent pharmacological functions (Shen et al. 2009). Resveratrol, known as trihydroxystilbene, is the most famous representative of this group and occurs in grapes and red wine (Fig. 13.8). Resveratrol initially attracted attention for the cardioprotective properties of red

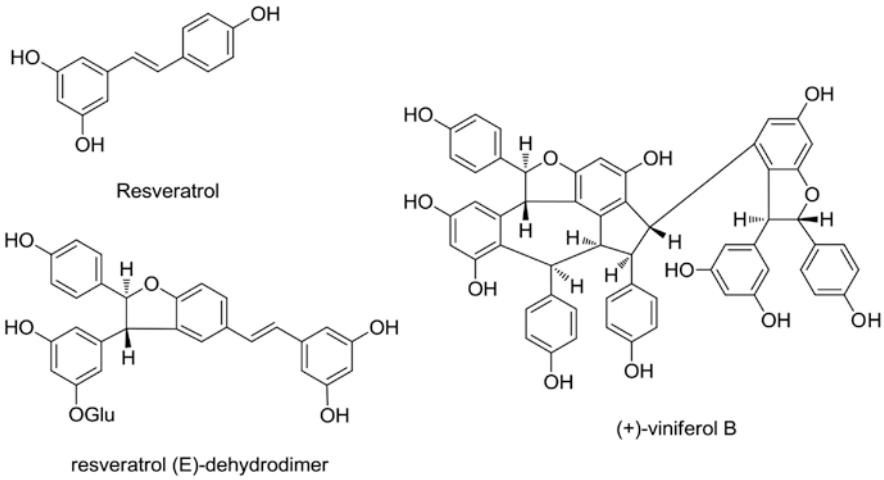

Fig. 13.8 Structures of typical stilbenes in the human diet

wine. Subsequently, it became a "star compound" from the pharmacological point of view for its potential to reduce cancer, cardiovascular disease, and ischemic injuries (Baur and Sinclair 2006). In addition, resveratrol can polymerize to form oligomers with intricate structures, complex configuration, and a variety of skeletons. The resveratrol oligomers, resveratrol (*E*)-dehydrodimer 11-*O*-β-D-glucopyranoside and (+)-viniferol B, were isolated from the grape *Vitis vinifera* (Waffo-Teguo et al. 2001; Yan et al. 2002) (Fig. 13.8).

13.2.5 Miscellaneous Dietary Polyphenols

Besides the above-mentioned types of natural phenolic substances, many other dietary polyphenols also contribute to the human health (Fig. 13.9). Curcumin, the principal component of turmeric and curry powder, is widely used as a spice and coloring agent in food and is considered to be a potential candidate for the prevention and/or treatment of some human diseases (Hatcher et al. 2008). Ginger is a condiment for various foods and beverages and has a long medical history in many folk medicines. To a certain extent, its traditional uses are attributed to the phenolic constituent gingerol (Shukla and Singh 2007). Rosmarinic acid, an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, occurs in lemon balm, oregano, rosemary, sage, thyme, and peppermint (Lee 2010). Ellagic acid is a dimer of gallic acid bearing two lactone rings and mainly occurs in berries, fruits, and nuts (Vattem and Shetty 2005). Avenanthramides, exemplified by A1 and A2, are a group of N-containing polyphenols present mainly in oats (Dimberg et al. 1993). Capsaicin is the irritant constituent of chili pepper that produces the sensation of burning (Hayman and Kam 2008).

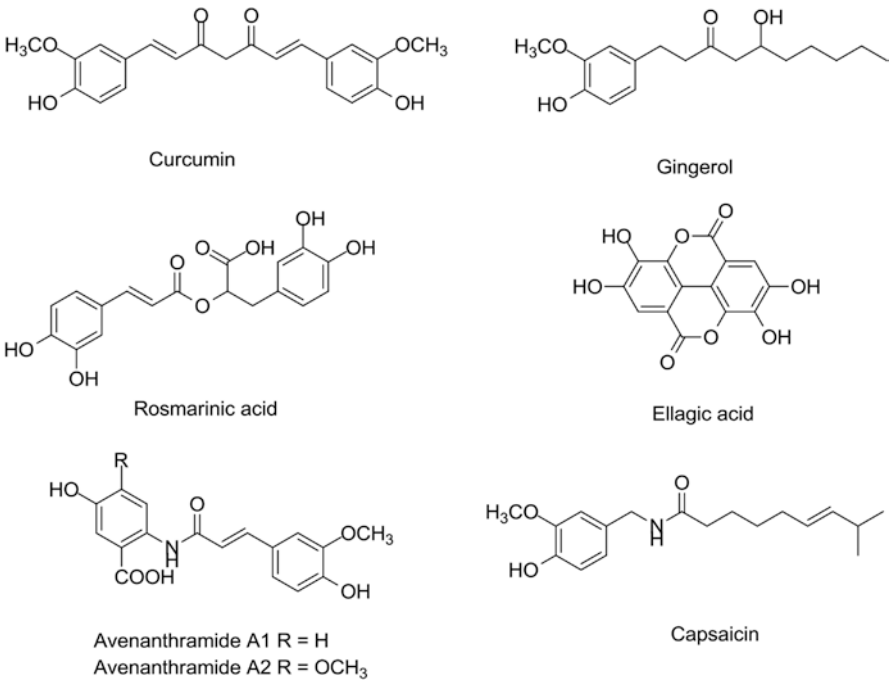

Fig. 13.9 Chemical structures of miscellaneous dietary polyphenols

13.3 Roles of Dietary Polyphenols in Disease Prevention

13.3.1 Inhibition of Oxidative Stress

Oxidative stress is a disturbance in the balance between the production of ROS and antioxidant defenses (Betteridge 2000). ROS can be defined as any chemical species containing unpaired electrons and so increases the chemical reactivity of an atom or molecule. Common ROS in humans includes hydroxyl ($^{\bullet}OH$), superoxide ($^{\bullet}O_2^-$), nitric oxide ($NO^{\bullet}$), peroxyl ($RO_2^{\bullet}$), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and ozone (O_3) (Aruoma 1998). The balance between cellular ROS generation and antioxidant defense capacity determines the status of oxidative stress. Once cellular antioxidant defense systems are deficient, ROS could damage cell components (proteins, lipids, and DNA) and become an underlying triggering factor for many human diseases. The mechanisms of cellular damage by oxidative stress have been reviewed (Aruoma 1998; Finkel and Holbrook 2000) and summarized in Fig. 13.10. There is substantial support for a role of oxidative stress in pathogenesis of diabetes (Baynes 1991), cancer (Reuter et al. 2010), cardiovascular disease (CVDs) (Madamanchi et al. 2005), and neurodegenerative diseases (Finkel and Holbrook 2000).

In order to neutralize the deleterious effects of ROS and alleviate oxidative stress, various antioxidant strategies have evolved by increasing the endogenous antioxidant

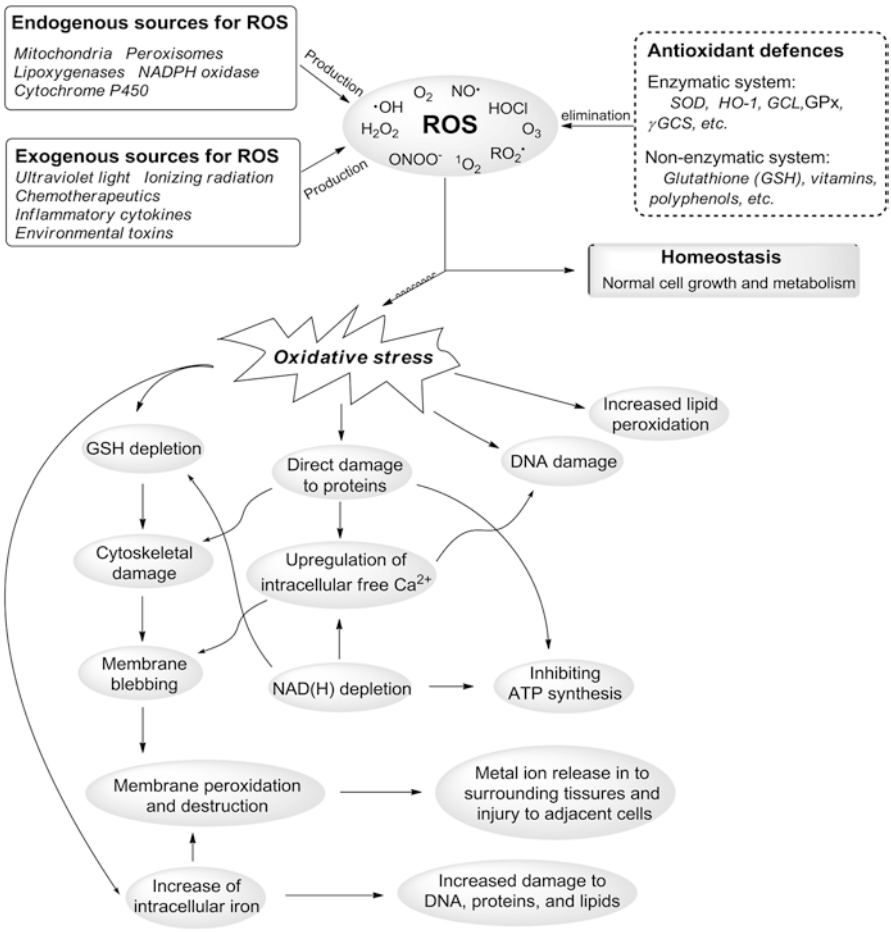

Fig. 13.10 Antioxidant defenses, sources of reactive oxygen species (ROS), and cellular damages

enzyme defenses and by enhancing the nonenzymatic defenses through dietary or pharmacological methods. Many studies indicate that polyphenols directly scavenges ROS in vitro (Russo et al. 2000; Visioli et al. 1998; Lodovici et al. 2001; Lu and Foo 2000). However, the high levels of some polyphenols required to exert such direct antioxidant effects are difficult to achieve in vivo due to their limited bioavailability (Martin and Appel 2010). Lower concentrations of polyphenols that occur in vivo activate endogenous cytoprotective antioxidant enzymes to facilitate the elimination of ROS and counteract ROS-induced damage. The primary mechanisms of dietary polyphenols against oxidative stress are illustrated in Fig. 13.11.

Cytoprotective antioxidant enzymes are commonly regulated by antioxidant response elements (ARE) promoter regions and these enzymes are represented by γ-glutamylcysteine synthetase (γGCS), glutamate cysteine ligase (GCL), glutathione

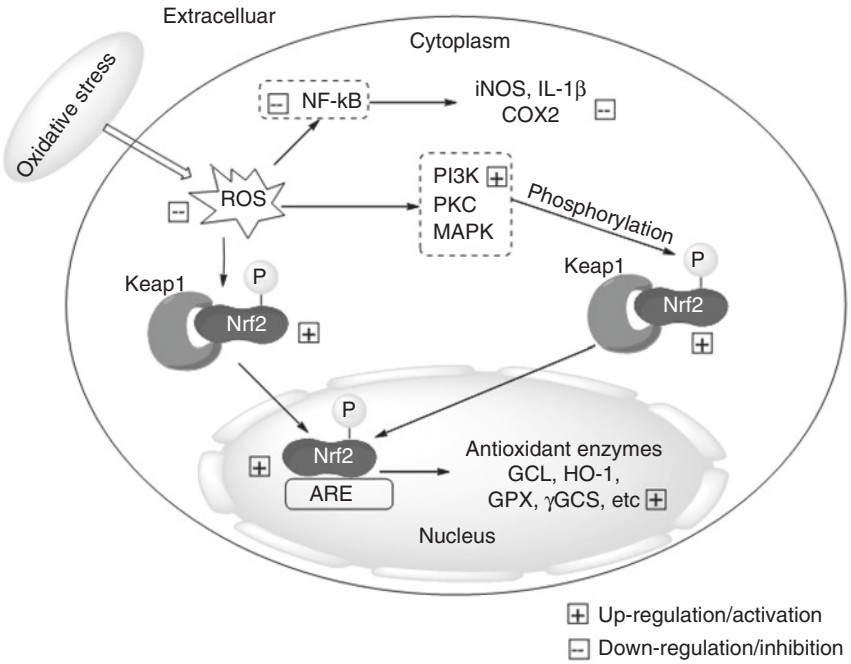

Fig. 13.11 The primary mechanism of action of dietary polyphenols against oxidative stress

peroxidase (GPX), thioredoxin (TRX), thioredoxin reductase (TRXR), peroxiredoxin (PRX), and heme oxygenase-1 (HO-1) that maintain cellular glutathione and TRX levels and reduce levels of ROS (Lau et al. 2008). This process is regulated through multiple cellular signaling pathways. Nrf2 plays an important role in maintaining cellular redox homeostasis by regulating these ARE-bearing genes. Under normal physiological conditions, Nrf2 is sequestered by Kelch-like ECH-associated protein 1 (Keap1) in the cytosol and limits the expression of antioxidant genes. Upon exposure of cells to dietary polyphenols, Nrf2 is activated and translocates to the nucleus, binds to the ARE sequence to activate cellular antioxidant enzymes, which remove the overproduced ROS (Shen et al. 2014). Dietary polyphenols inhibit oxidative stress through interacting with mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), phosphatidylinositol 3-kinase (PI3K), and RNA-dependent protein kinase-like endoplasmic reticulum kinase (PERK), because these kinases activate Nrf2 by phosphorylation (Jeong et al. 2006). Furthermore, dietary polyphenols are capable of suppressing NF-κB activation; inhibiting the expression of inducible nitric oxide synthase (iNOS), interleukin (IL)-1β, and COX2; and thus reducing cellular adoptive responses.

A growing body of evidences supports a role for dietary polyphenols, such as resveratrol, curcumin, EGCG, quercetin, and caffeic acid, in the inhibition of oxidative stress. EGCG upregulates ARE-mediated antioxidant gene expression and enhances cellular antioxidant capacity through activation of MAPK proteins

(Chen et al. 2000). Quercetin promotes Nrf2-mediated transcription activity, enhances Nrf2 stabilization, upregulates endogenous antioxidant glutathione (GSH) level, and increases expressions of cellular antioxidant enzymes, such as superoxide dismutase (SOD), GPX, and γGCS (Molina et al. 2003; Scharf et al. 2003; Tanigawa et al. 2007). Quercetin also has the potential to inhibit NF-κB activation (Abramov et al. 2005). Resveratrol increases the transcriptional activity of Nrf2, upregulates the expression of HO-1 and γGCS, and thereby protects cells against oxidative stress-related insults (Ungvari et al. 2007; Csiszar et al. 2011). Like resveratrol, curcumin activates Nrf2 and cellular antioxidant enzymes regulation. The potency of dietary polyphenols on oxidative stress was verified in several studies, which provide evidence that dietary polyphenols can prevent oxidative stress-related NCDs, such as cancer, inflammation, CVDs, and diabetes.

13.3.2 Inhibition of Inflammation

Inflammation is a complex biological response of vascular tissues to harmful stimuli and is also a reaction of the microcirculation in dealing with the movement of serum proteins and leukocytes from the blood to the extravascular space. It is a protective immunovascular response in humans that is regulated by many mediators, including the vasoactive amines, histamine and 5-hydroxytryptamin; the lipid-derived eicosanoids, prostaglandin E2 (PGE2), prostaglandin I2 (PGI2), leukotriene B4 (LTB4), and leukotriene C4 (LTC4); and several cytokines, such as tumor necrosis factor α (TNFα), IL-1β, IL-6, and IL-10 (Santangelo et al. 2007). Inflammation is a crucial pathogenic factor for obesity, diabetes, CVDs, neurodegenerative diseases, and cancer (Coussens and Werb 2002; Libby et al. 2002; Dandona et al. 2004; Minghetti 2005). Dietary polyphenols play important role in the prevention of inflammation (Yoon and Baek 2005; Santangelo et al. 2007) at least in part through interacting with various anti-inflammatory targets (Fig. 13.12).

13.3.2.1 Inhibition of Arachidonic Acid Pathway

Arachidonic acid (AA), which is released by membrane phospholipids via phospholipase A2 (PLA2) cleavage, is further metabolized by the COX pathway into prostaglandins (PGs) and thromboxane A2 (TXA2) or by the LOX pathway to leukotrienes (LTs), hydroperoxyeicosatetraenoic acids (HpETEs), and hydroxyeicosatetraenoic acids (HETEs). These eicosanoids play important roles in the regulation of the inflammatory process. The COX enzymes exist in two major isoforms, COX-1 and COX-2, and the latter is an inducible enzyme that highly expressed in cells stimulated by pro-inflammatory cytokines. The LOX isoenzymes, 5-, 12-, and 15-LOXs, are involved in the generation of LTs, HpETEs, and HETEs. It is important to note that inhibiting the eicosanoid-generating enzymes, PLA2, COX, and LOX, is an effective approach for limiting the inflammatory response and reducing

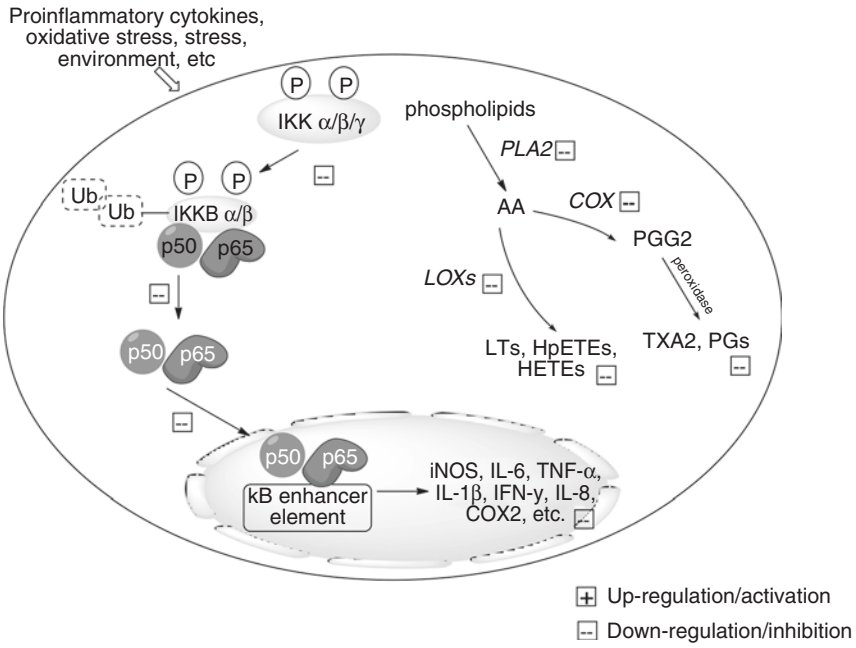

Fig. 13.12 The molecular mechanism of action of dietary polyphenols in inflammation prevention

inflammation. Dietary polyphenols inhibit these above-mentioned enzymes at both the transcriptional and protein level and thus reduce the productions of AA, PGs, and LTs (Table 13.2).

13.3.2.2 Modulation of Nitric Oxide (NO) Synthesis

$NO^{\bullet}$ is a cellular mediator synthesized from L-arginine by the NOS family that includes the endothelial (eNOS), neuronal (nNOS), and inducible NOS isoforms. A physiologic level of $NO^{\bullet}$, synthesized by eNOS and nNOS, is essential for normal organ functions, while overproduced $NO^{\bullet}$ induced by iNOS provokes an inflammatory response. Thus, some polyphenols that are capable of suppressing iNOS enzyme expression and iNOS activity (Table 13.2) can prevent inflammation.

13.3.2.3 Regulation of Cytokines

Cytokines are small proteins released by cells and are key mediators of local and intercellular communications required for the cellular immune response process to inflammation. They include interferon gamma (IFN-γ), interleukins (ILs-1β, 2, 4, 6, 8, 10), and TNFα. The cytokines are classified into two groups, the pro-inflammatory

Table 13.2 Protection and mechanisms of action of some dietary polyphenols against inflammation

Dietary polyphenols	Protective effect and their mechanisms of action	Reference
Caffeic acid	Decreased the production of IL-1β in activated human whole blood cells	Miles et al. (2005)
Catechin, epicatechin gallate, epigallocatechin, EGCG	Inhibited expression of COX-2 in human mammary epithelial MCF-10A cells and mouse skin induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate	Kundu et al. (2003)
	Inhibited iNOS transcription and activity in mouse peritoneal cells treated by LPS and IFNγ	Chan et al. (1997)
	Decreased the production of IL-1β and enhanced the production of IL-10 in human blood cells	Crouvezier et al. (2001)
Apigenin, quercetin, kaempferol, myricetin, genistein, resveratrol	Inhibited NO production in LPS/interferon γ-activated C6 astrocytes	Soliman and Mazzio (1998)
	Inhibited the production of iNOS, TNF-α, and formation of NO in LPS-activated macrophages	Wadsworth and Koop (1999)
	Inhibited production of NF-κB and IL-8 in human synovial cells	Sato et al. (1997)
	Inhibited IκBα phosphorylation and downregulated production of TNF-α and IL-1β	Comalada et al. (2005)
	Inhibited phorbol ester-mediated induction of COX-2 transcription and activity in the human mammary 184B5/HER and oral epithelial MSK- Leuk1 cells	Subbaramaiah et al. (1998)
	Inhibited NF-κB activation and reduced TNF-α, IL-1β, IL-6, and COX-2 gene expression	Gonzales and Orlando (2008)
Curcumin	Inhibited the release of arachidonic acid through blocking the phosphorylation of cPLA2, decreasing the COX-2 expression, and 5-LOX catalytic activities in lipopolysaccharide-stimulated RAW cells and/or A23187-stimulated HT-29 cells	Hong et al. (2004)
	Inhibited NF-κB activation and decreased gene expression of TNF-α and IL-1β	Gonzales and Orlando (2008)

cytokines, IL-1β, IL-2, IL-6, IL-8, IFN-γ, and TNFα, and the anti-inflammatory cytokines, IL-4 and IL-10. Overexpression of pro-inflammatory cytokines and deficiency in the production of anti-inflammatory cytokines contribute to inflammation-related diseases (Dinarello 2000). Some dietary polyphenols demonstrate potential in the treatment of inflammatory diseases through interfering with the formation and/or action of these cytokines (Table 13.2).

13.3.2.4 Inhibition of Nuclear Factor-Kappa B Pathway

Nuclear transcription factor κB is a ubiquitous factor that resides in the cytoplasm and can be activated by ROS, inflammatory stimuli, and carcinogenic factors. Upon activation, NF-κB induces the expression of its downstream genes that are involved

in the regulation of cell apoptosis, proliferation, invasion, metastasis, and inflammation. NF-κB is a mediator of cancer, atherosclerosis, diabetes, and inflammatory diseases. NF-κB-mediated genes that are related to inflammation include those encoding pro-inflammatory cytokines, COX-2, iNOS, and matrix metalloproteinases (MMPs) (Tak and Firestein 2001). The mechanism of action of the NF-κB signaling pathway is summarized in Fig. 13.12. NF-κB is a dimmer consisting of a p50 subunit and a trans-activating subunit p65. Under the basal conditions, NF-κB is sequestered in the cytoplasm and regulated by the κB (IκBs) inhibitors, IκBα, IκBβ, and IκBγ, which inactivate NF-κB via binding with p50-p65 heterodimer. Phosphorylation of IκBs is a key step in NF-κB activation, which is mediated by the IκB kinase (IKK). After phosphorylation by IKK, IκBα is ubiquitinated and subjected to degradation by 26 proteasome, resulting in the release of NF-κB. The released NF-κB dimer then translocates into the nucleus and induces the expression of its downstream genes that contain κB enhancer elements. Therefore, inhibition of NF-κB is regarded as a useful approach for the treatment of inflammatory disorders (Karin et al. 2004), and some polyphenols inhibit inflammatory responses through targeting NF-κB pathway (Table 13.2).

13.3.3 Cancer Prevention

Despite progress in early diagnosis and treatment, cancer is still a major public health concern around the world. Carcinogenesis is a complex multiple-step process that is composed of three typical stages: (1) initiation, a rapid phase that is produced by the exposure and interaction of cells with carcinogens; (2) promotion, a relatively lengthy and revisable phase with abnormal cell expansion caused by the altered expression of genes whose products are related with hyperproliferation, tissue remodeling, and inflammation; and (3) progression, the final state with uncontrolled growth of the cancer cells possessing an increasing ability of invasiveness, metastasis, and angiogenesis (Surh 2003) (see Fig. 13.13). Intervention in the carcinogenic processes with natural or synthetic chemicals, termed as "chemoprevention," is an effective approach to inhibit the incidence of cancer. It has been estimated that 35% of cancer prevention might be related to life style behaviors, mainly related to consumption of a healthy diet (Manson 2003). Polyphenols are important for lowering the incidence of some cancers by preventing carcinogenesis. Furthermore, dietary polyphenols are a promising group of chemopreventive agents since they have low toxicity and are in widespread use.

Any of the exogenous or endogenous carcinogens, inflammatory cytokines, and tumor promoters can activate the carcinogenesis by regulating the transcription factors, NF-kB and AP-1; the anti-apoptotic proteins, Akt, Bcl-2, and Bcl-XL; the pro-apoptotic proteins, caspases and PARP; the protein kinases, IκB kinase (IKK), c-Jun N-terminal Kinase (JNK), and MAPK; the cell cycle proteins, cyclins and CDK; and growth factor signaling pathways (Aggarwal and Shishodia 2006). In the initiation phase, carcinogens interact with cellular DNA and cause damage; hence, blocking

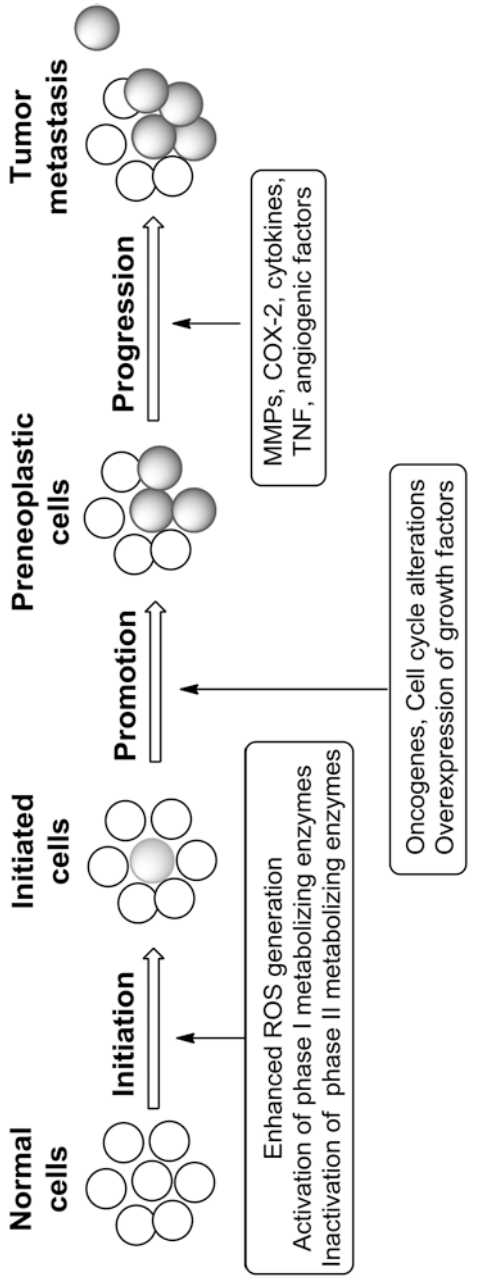

Fig. 13.13 The multiple-step process for carcinogenesis

these genotoxic damages can be an effective approach for cancer prevention, which could be achieved by eliminating ROS and inducing phase II conjugating enzymes. In the promotion stage, inhibiting cell proliferation is a useful strategy, such as by modulating cell cycle arrest and apoptosis. In the progression stage, interrupting angiogenesis and/or preventing progression of malignant cells to metastasis and invasiveness are particularly important (Ramos 2008). Collectively, chemicals that inhibit or reverse carcinogen-induced cellular alterations have the potential to be effective chemopreventive agents. A great deal of evidence indicates that dietary polyphenols exert a protective function in the multiple-step process of carcinogenesis by acting with various cellular targets that regulate carcinogen activation/detoxification by phase I or phase II metabolizing enzymes, DNA repair, cell cycle progression, cell proliferation and apoptosis, angiogenesis, and metastasis (Manson 2003; Surh 2003; Aggarwal and Shishodia 2006). The possible mechanisms by which dietary polyphenols inhibit carcinogenic processes are illustrated in Fig. 13.14.

13.3.3.1 Effect on Phase I and Phase II Metabolizing Enzymes

Procarcinogens can be activated by the phase I metabolizing enzymes, cytochrome P450 or CYP P450 isoforms, detoxified by the phase II metabolizing enzymes, nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase (NQO1), quinone reductase (QR), and glutathione S-transferase (GST), through glucuronidation, sulfation, acetylation, and conjugation, and then eliminated from the human body. Thus, inhibition of procarcinogen activation and promotion of their elimination by targeting phase I and phase II enzymes are effective strategies in preventing the initiation stage. Myricetin, apigenin, quercetin, kaempferol, and EGCG are

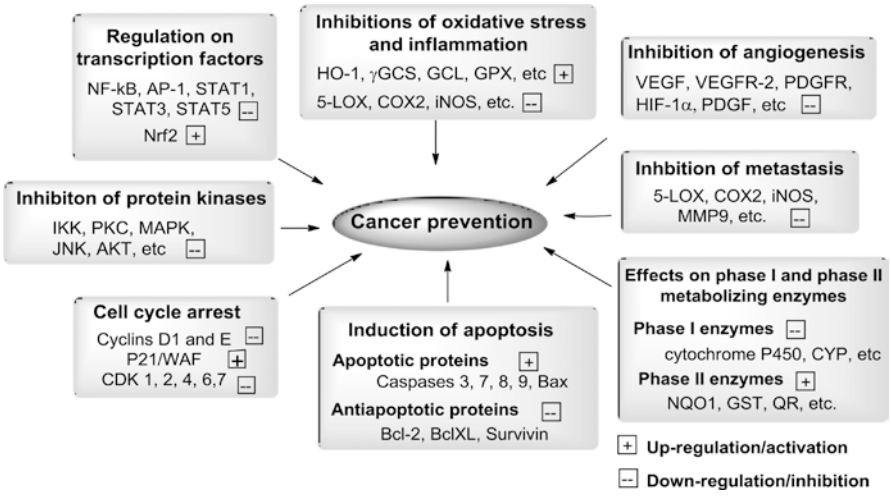

Fig. 13.14 The molecular mechanism of action of dietary polyphenols in cancer prevention

potent inhibitors of CYP1A1, a key enzyme involved in the metabolism of xenobiotics (Schwarz and Roots 2003). Chlorogenic acid, which is rich in plum and cherry, increases the activity of the phase II detoxifying enzymes, GST and NQO1, in mouse epidermal cells (Shan et al. 2009). Resveratrol, curcumin, and EGCG upregulate phase II metabolizing enzymes (Kou et al. 2013).

13.3.3.2 Inhibition of Oxidative Stress and Inflammation

ROS damage proteins, DNA, and RNA, as well as oxidize fatty acids in cell membranes, and thus increase the opportunity for mutations. ROS also activate several transcription factors, such as NF-κB, AP-1, p53, peroxisome proliferator activator receptor γ (PPARγ), and Nrf2. These transcription factors subsequently modulate the expression of over 500 different genes, including those encoding growth factors, inflammatory cytokines, chemokines, and anti-inflammatory molecules (Reuter et al. 2010). Therefore, ROS-induced oxidative stress can lead to chronic inflammation, which is in turn associated with an increased risk of human cancers (Bartsch and Nair 2006). Inhibition of oxidative stress and inflammation is considered an important mechanism for chemopreventive agents. The preventive actions of typical dietary polyphenols against oxidative stress and inflammation are discussed in Sects. 13.3.1 and 13.3.2.

13.3.3.3 Inhibition of Nuclear Factor-Kappa B Transcription Factor

NF-kB, which can be activated by carcinogens, ROS, and inflammatory stimuli, translocates to the nucleus where it induces more than 200 genes that are related to the regulation of apoptosis, cell proliferation, invasion, metastasis, inflammation, and chemoresistance. Some of NF-kB target genes, such as cyclin D1 (cell cycle regulator), Bcl-2 and Bcl-XL (anti-apoptotic proteins), MMPs, (modulator of metastasis), and vascular endothelial growth factor (VEGF, which is involved in angiogenesis), play crucial roles in the development of aggressive cancers (Aggarwal and Shishodia 2006). Inhibition of NF-kB is beneficial for blocking carcinogenesis. Dietary polyphenols, such as ECGC, curcumin, resveratrol, quercetin, genistein, and ellagic acid can act as NF-kB inhibitors (Aggarwal and Shishodia 2006).

13.3.3.4 Inhibition of Activator Protein-1 Transcription Factor

AP-1 is a transcription factor produced by either homo- or heterodimers of the proteins from the Jun and Fos family (Eferl and Wagner 2003). AP-1 activation induces the expression of its target genes, such as cyclin D1, p53, and p21 that are involved in apoptosis and proliferation. Furthermore, AP-1 promotes tumor metastasis through inducing the transition of tumor cells form an epithelial to mesenchymal morphology. It has been shown that AP-1 is highly activated in most cancer cell

lines (Fresco et al. 2006). Catechins, theaflavin, quercetin, resveratrol, and curcumin can inhibit tumor metastasis through AP-1 inhibition (Aggarwal and Shishodia 2006; Fresco et al. 2006).

13.3.3.5 Modulation of Mitogen-Activated Protein Kinases

The MAPK cascades are comprised of extracellular signal-regulated protein kinases (ERKs), c-Jun N-terminal kinases/stress-activated protein kinases (JNKs/SAPKs), and p38 kinases, which are activated by the growth-inducing tumor promoter, epidermal growth factor (EGF), and platelet-derived growth factor (PDGF), or stress-related tumor promoters (Aggarwal and Shishodia 2006). The MAPK pathway consists of a cascade in which MAP3K activates MAP2K that then activates ERK, JNK, and p38. These signaling cascades can interplay with other signaling pathways, such as NF-kB, AP-1, and Nrf2, leading to the regulations of cell proliferation, differentiation, survival, and migration (Seger and Krebs 1995). The dietary polyphenols apigenin, curcumin, resveratrol, as well as green tea polyphenols are modulators of the MAPK pathway.

13.3.3.6 Cell Cycle Arrest

In cancer cells, normal cell growth and behavior are lost due to the aberrant cell cycle regulation. Major control switches of the cell cycle are the cyclins and the CDKs, whose dysfunction is associated with carcinogenesis (Semczuk and Jakowicki 2004). Dietary polyphenols inhibit cell proliferation during different cell phases. Curcumin arrests human umbilical vein endothelial cells at the G0/G1 and/or G2/M phases by upregulating CDK inhibitors (p21waf1/Cip1 and p27Kip1) and downregulating cyclin B1 (Park et al. 2002). EGCG blocks MCF-7 cell cycle progression at the G1 phase by inhibiting CDKs 2 and 4, inducing the expression of p21waf1/Cip1 and p27Kip1 genes, and reducing the expression of cyclin D1 (Liang et al. 1999).

13.3.3.7 Induction of Apoptosis

Apoptosis is a programmed cell death that establishes a natural balance between cell death and cell renewal in biological organs. It is regulated by the apoptotic proteins, caspases and Bax, and by the antiapoptotic proteins, Bcl-2, Bcl-XL, and survivin. However, the balance between survival and apoptosis favors the former in cancer cells. Accordingly, apoptosis induction is considered as a preventive approach against cancer. Many dietary polyphenols, including quercetin, EGCG, apigenin, ellagic acid, curcumin, and resveratrol, inhibit carcinogenesis by inducing apoptosis (Surh 2003). For example, curcumin suppresses the constitutive expression of Bcl-2 and Bcl-XL, activates caspase-7 and caspase-9, and induces poly (ADP-ribose) polymerase (PARP) cleavage in human multiple myeloma cell lines (Bharti et al. 2003).

13.3.3.8 Inhibition of Angiogenesis

Angiogenesis is characterized by the formation of new blood vessels from a pre-existing microvascular network and represents a fundamental step in tumor growth. Angiogenesis is regulated through the modulation of proliferation and gene expression by endothelial cells. Inhibition of angiogenesis is a potential target for the control of tumor growth, invasion, and metastasis (Gourley and Williamson 2000). VEGF, VEGF receptor-2 (VEGFR-2), PDGF, PDGF receptor (PDGFR), hypoxia-inducible factor 1α (HIF-1α), and matrix metalloproteases (MMPs) regulate angiogenesis. Resveratrol inhibits VEGF-induced angiogenesis (Lin et al. 2003). Quercetin, myricetin, kaempferol, and galanin suppress the VEGF-stimulated tubular formation and inhibit U937 activated monocytic cell adhesion in the human umbilical vein endothelial cells (Kim et al. 2006).

13.3.4 Cardiovascular Disease Prevention

Many studies suggest that consumption of polyphenols limits the incidence of CVDs, such as hypertension, atherosclerosis, and strokes (Stoclet et al. 2004; Rasmussen et al. 2005; Zern and Fernandez 2005; Borriello et al. 2010). The polyphenols primarily elaborate their cardioprotective functions (Curin and Andriantsitohaina 2005; Borriello et al. 2010) by means of the following mechanisms.

13.3.4.1 Improvement of Serum Cholesterol and Triglyceride Profiles

Oxidation of low-density lipoprotein (LDL) triggers the release of pro-inflammatory mediators and induces endothelial injury during atherogenesis (Reaven and Witztum 1996). Polyphenols are able to protect LDL from peroxidation by virtue of their potent antioxidant capacity. Tea catechins suppress the oxidative modification of LDL (Miura et al. 1994; Yokozawa and Dong 1997). Consumption of red wine polyphenols reduces the susceptibility of LDL to oxidation in human studies (Nigdikar et al. 1998). Polyphenols exert cardiovascular protection by regulating lipid metabolism. Accumulation of cholesterol is a crucial step for atherogenesis and resveratrol prevents this in human macrophages (Sevov et al. 2006). Catechins decrease cholesterol and triglyceride levels in the rat aorta (Miura et al. 2001). Administration of hesperidin and naringenin for 2–6 weeks reduces the levels of total plasma cholesterol, LDL cholesterol, and triglycerides in hyperlipidemic rat models (Monforte et al. 1995; Lee et al. 1998).

13.3.4.2 Inhibition of Platelet Aggregation and Thrombosis

Platelet aggregation contributes to the development of atherosclerosis and its inhibition benefits cardiovascular health. Platelets release pro-inflammatory mediators, such as TXA2, platelet-activating factor (PAF) and serotonin that have important

roles in atherogenesis. The major mechanisms by which polyphenols prevent thrombosis are related to the inhibition of thromboxanes (TXs), COX, and LOX. The role of dietary polyphenols in regulating thrombosis was shown in in vitro and in vivo studies (Nardini et al. 2007). Resveratrol is a potent inhibitor of COX1, which is involved in the synthesis of TXA2, an inducer of the platelet aggregation (Szewczuk et al. 2004; Pirola and Fröjdö 2008). Hydroxytyrosol inhibits the production of TXB2 (Petroni et al. 1995), and epicatechin is a potent inhibitor of 5-LOX (Schewe et al. 2002). Red wine increases the bleeding time and inhibits platelet aggregation and thrombosis in rats (Wollny et al. 1999) and in dogs (Demrow et al. 1995).

13.3.4.3 Improvement of Endothelial Function

Impaired endothelial function is associated with the incidence of CVDs. A healthy vascular endothelium synthesizes and releases endothelium-derived relaxing factors, such as $NO^{\bullet}$, which is an important mediator that promotes endothelium-dependent vasorelaxation and limits the movement of atherogenic plasma proteins into the arterial wall. Resveratrol increases the activities of eNOS and iNOS, whose accumulation in blood vessel leads to vasodilatation through augmented $NO^{\bullet}$ (Das et al. 2005). The endothelium-dependent vasorelaxing properties of polyphenols, including anthocyanins, soy isoflavones, quercetin, and resveratrol, were shown in isolated rat or rabbit aorta and in female macaques (Chen and Pace-Asciak 1996; Honore et al. 1997; Andriambeloson et al. 1998). Furthermore, a cross-sectional study of 218 women reports that long-term regular intake of black tea lowers blood pressure. Besides releasing relaxing factors, vascular endothelium also produces potent contracting factors. Endothelin-1 (ET-1) is a potent vasopressor released by endothelial cells. Resveratrol downregulates the transcription of ET-1 (Liu et al. 2003), which is implicated in prevention of hypertension. These cardioprotective effects of resveratrol are related to improved endothelial function.

13.3.4.4 Inhibition of Oxidative Stress and Inflammation

As described in Sects. 13.3.1 and 13.3.2, oxidative stress and inflammation contribute to the development of CVDs. Dietary polyphenols inhibit oxidative stress and inflammation through various mechanisms and so protect human cardiovascular health from injury induced by oxidative stress and inflammation.

13.3.5 Diabetes Prevention

Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia and is classified into type 1 diabetes and type 2 diabetes. Type 2 diabetes represents 90% of diabetic cases worldwide and is associated with insulin resistance, impaired insulin signaling, pancreatic β-cell dysfunction, oxidative stress, and abnormal

glucose metabolism (Bahadoran et al. 2013). Diabetes causes many complications, including coronary artery disease, cerebrovascular disease, nephropathy, neuropathy, retinopathy, etc. Dietary polyphenols lower the risk of the diabetes and are regarded as an effective strategy for the prevention of diabetes (Montonen et al. 2004; Pandey and Rizvi 2009; Bahadoran et al. 2013). The primary mechanisms of dietary polyphenols against diabetes are summarized (Bahadoran et al. 2013) and presented in Fig. 13.15.

13.3.5.1 Decreasing the Intestinal Absorption of Glucose

The main targets of dietary polyphenols on disturbing intestinal absorption of glucose include α-glucosidase, α-amylase, and Na^+/glucose cotransporters. α-Glucosidase and α-amylase are two key enzymes involved in the conversion of complex dietary carbohydrates to glucose in the gut, and inhibition of these two enzymes significantly reduces blood glucose levels. Dietary polyphenols, including flavonoids, tannins, resveratrol, and phenolic acids, have hypoglycemic effects through inhibition of α-glucosidase and α-amylase (McDougall et al. 2005; Kerem et al. 2006; Tadera et al. 2006; Matsui et al. 2007; Kwon et al. 2008; Adisakwattana et al. 2009; Tundis et al. 2010). After hydrolysis of carbohydrates, glucose uptake is mediated by intestinal sodium-dependent glucose cotransporter (SGLT). EGCG,

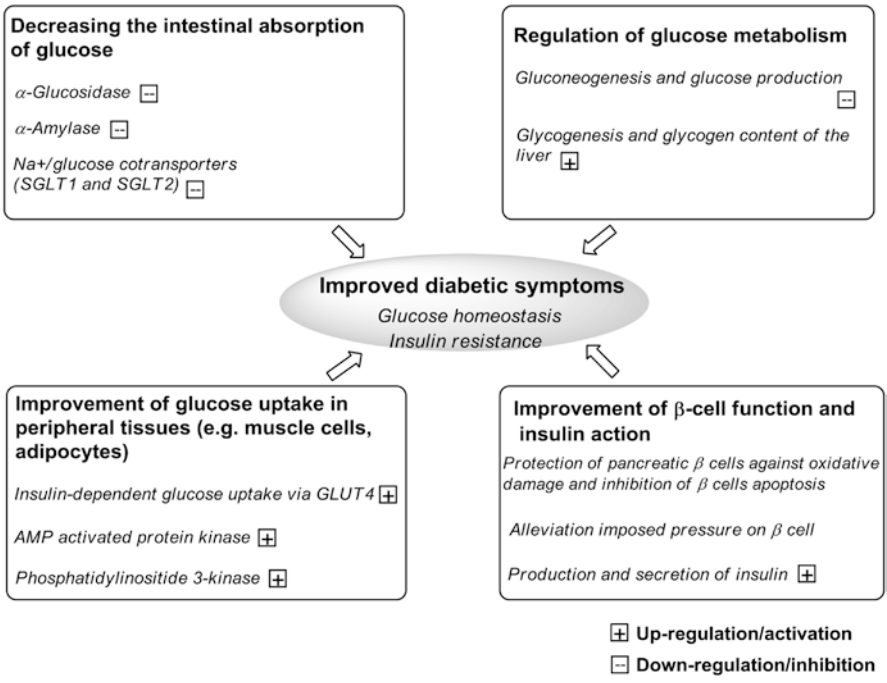

Fig. 13.15 The molecular mechanism of action of dietary polyphenols in diabetes prevention

(−)-epichatechingallate, (−)-epigallochatechin, phloridzin, chlorogenic acids, ferulic acids, caffeic and tannic acids, quercetin and apigenin, obstruct glucose absorption by inhibiting SGLT1 and/or SGLT2 (Welsch et al. 1989; Johnston et al. 2005). Phloridzin inhibits glucose reabsorption in the kidney (Dimitrakoudis et al. 1992).

13.3.5.2 Regulation of Glucose Metabolism

Dietary polyphenols improve glucose homeostasis through regulating glucose metabolism. EGCG displays insulinomimetic properties and represses hepatic glucose production by activating 5′-AMP-activated protein kinase (AMPK) that is involved in the inhibition of gluconeogenic enzymes (Waltner-Law et al. 2002; Collins et al. 2007). Administration of ferulic acid elevates hepatic glycogen synthesis and lowers the blood glucose levels in type 2 diabetic mice (Jung et al. 2007).

13.3.5.3 Improvement of Glucose Uptake in Peripheral Tissues

Some studies report that polyphenols increase glucose uptake in both insulin sensitive and non-insulin sensitive peripheral tissues. Caffeic acid promotes glucose uptake by myoblasts and adipocytes in rat (Cheng and Liu 2000; Hsu et al. 2000). Resveratrol improves glucose uptake in myoblasts by translocating the glucose transporter 4 (GLUT4), to the plasma membrane by activation of the AMPK pathway (Park et al. 2007). Phosphatidylinositol-3 kinase (PI3K) is a key molecular switch that mediates the metabolism of insulin, glucose transport, and GLUT-4 translocation. Oral administration of resveratrol increases GLUT4 expression in rat skeletal muscle by activating the PI3K pathway (Chi et al. 2007).

13.3.5.4 Improvement of β-Cell Function and Insulin Action

Genistein improves pancreatic β-cell proliferation and survival by inducing cyclin D1 protein expression and promoting glucose-induced insulin secretion in pancreatic β cells (Fu and Liu 2009; Fu et al. 2010). Resveratrol and anthocyanins protect β cells against oxidative stress and cell apoptosis and thus improve glucose tolerance (Zhang et al. 2010; Szkudelski and Szkudelska 2011). Epidemiological evidence suggests that consumption of coffee decreases the incidence of type 2 diabetes due to the inhibition of intestinal absorption of glucose (Van Dam and Feskens 2002).

13.3.6 Neurodegenerative Disease Prevention

Neurodegenerative diseases are characterized by progressive and selective loss of structure or function of neuronal systems. Typical neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral

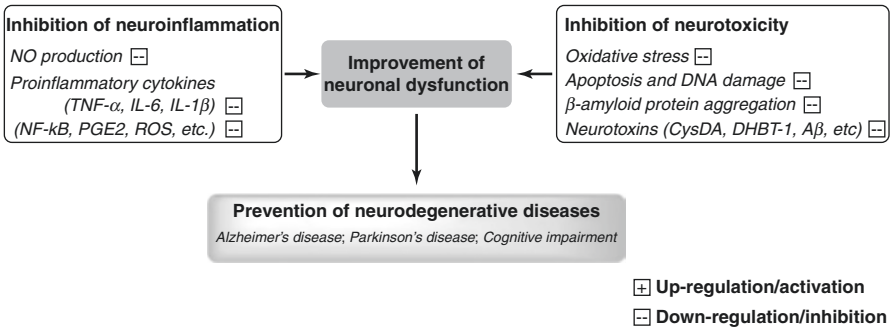

Fig. 13.16 Molecular mechanism of action of dietary polyphenols in neurodegenerative disease prevention

sclerosis (ALS), and Huntington's disease (HD) (Lin and Beal 2006). AD and PD afflict approximately 15% and 1% of the population aged over 65, respectively. Oxidative stress, neuroinflammation, glutamatergic excitotoxicity, and/or damage to brain macromolecules play critical roles in the progression of neurodegenerative diseases (Barnham et al. 2004). The potential capacity of polyphenols to act as antioxidant and anti-inflammatory agents suggest that consumptions of polyphenol-rich foods would halt the progression and protect the tissues against the underlying pathology of neurodegenerative diseases. The mechanisms and molecular targets of dietary polyphenols against neurodegenerative diseases were comprehensively discussed (Vauzour 2012) and are presented in Fig. 13.16.

Neuroinflammation contributes to the cascade of events leading to neuronal degeneration, microglial activation, astrogliosis, and lymphocytic infiltration. Activated resident glial leads to the invasion of circulating immune cells and the productions of TNF-α, IL-1β, IL-6, $NO^{\bullet}$, PGE2, and ROS. Dietary polyphenols predominantly utilize four strategies to counteract neuroinflammation and inflammation-induced neural cell injuries (Vauzour 2012):

1. Inhibition of IL-1β and TNF-α release from neuroglial cells as shown by epicatechin and catechin induced inhibition of TNF-α production (Vafeiadou et al. 2009).
2. Inhibition of iNOS induction and subsequent $NO^{\bullet}$ production in response to glial activation as shown by quercetin induced decreases in $NO^{\bullet}$ production and iNOS gene expression in microglia (Kao et al. 2010).
3. Inhibition of NADPH oxidase and ROS generation in activated glia, where activation of NADPH oxidase boosts the production of $^{\bullet}O_2^-$ and the release of pro-inflammatory cytokines. Of note, ferulic acid, caffeic acid, tyrosol, and vanillic acid inhibit NADPH oxidase activity.
4. Downregulation of pro-inflammatory transcription factors such as NF-κB, which regulates some glial and neuronal signaling pathways, such as the MAPK cascade. It is important to note that EGCG, resveratrol, and curcumin inhibit NF-κB and modulate the MAPK and PKC pathways (Ramassamy 2006).

Inhibition of neurotoxicity is an additional pathway by which dietary polyphenols limit progression of neurodegenerative diseases. β-Amyloid (Aβ) is a small protein produced by cleavage of amyloid precursor protein under the catalysis of β-secretase and is implicated in the pathogenesis of AD. Oral administration of EGCG for 6 months inhibits Aβ pathology and improves cognitive function (Rezai-Zadeh et al. 2008). Treatment with ferulic acid protects mice against Aβ peptide toxicity induced by intracerebral injection of β-amyloid peptide (Yan et al. 2001) and ferulic acid and tannic acid depressed AD-like pathology in rats through β-secretase inhibition (Mori et al. 2012, 2013). Furthermore, dietary polyphenols, such as anthocyanins, curcumin, resveratrol, and catechins, obstruct the initiation and progression of AD by inhibiting oxidative stress, cell apoptosis, DNA damage, and β-amyloid aggregation in neurocytes (Mercer et al. 2005; Bastianetto et al. 2007; Darvesh et al. 2010). 5-S-cysteinyl-dopamine (CysDA) is an endogenous neurotoxin involved in the progression of PD. Hesperetin and (+)-catechin inhibit the formation of CysDA (Vauzour et al. 2007). Dietary polyphenols, such as pelargonidin, quercetin, hesperetin, caffeic acid, tyrosol, p-Coumaric acid, and catechins, protect neuronal cells against CysDA-induced toxicity (Vauzour et al. 2010).

13.3.7 Obesity Prevention

Obesity is a complex multifactorial disease associated with unhealthy lifestyle behaviors and environmental factors, as well as genetic factors. Currently, it is a major threat to public health and increases the incidences of diabetes and cardiovascular, pulmonary, and metabolic diseases. Dietary and behavioral modification programs are commonly adopted approaches for reducing energy intake and increasing expenditure to relieve the symptoms of obesity. Some reviews have comprehensively summarized the preventive or therapeutic potentials of dietary polyphenols against obesity (Lin and Lin Shiau 2006; Meydani and Hasan 2010; Wang et al. 2014). Catechins, resveratrol, and curcumin alleviate obesity and obesity-related chronic diseases. The primary mechanisms of dietary polyphenols against obesity have been summarized (Wang et al. 2014) and are presented in Fig. 13.17.

Catechins are the predominant bioactive constituents of green tea and constitute approximately 35% of its dry weight. The primary catechins are EGCG, (−)-epicatechin, (−)-epicatechin-3-gallate, and (−)-epigallocatechin. EGCG comprises more than 40% of green tea catechins (Chen et al. 2001). Herein, we discuss EGCG as an example to illustrate the protective effect and mechanisms of catechins and green tea against obesity. Firstly, consumption of tea and EGCG lowers the food intake through enhancement of cholecystokinin production (Liao et al. 2001). EGCG inhibits preadipocyte proliferation through induction of cell apoptosis and cell cycle arrest (Hung et al. 2005) and suppresses the adipocyte differentiation and thus blocks adipogenesis in 3T3-L1 preadipocytes (Furuyashiki et al. 2004; Chan et al. 2011). Inhibition of adipocyte differentiation by EGCG is associated with the down-regulations of the adipocyte differentiation regulators, PPARγ and CCAAT/

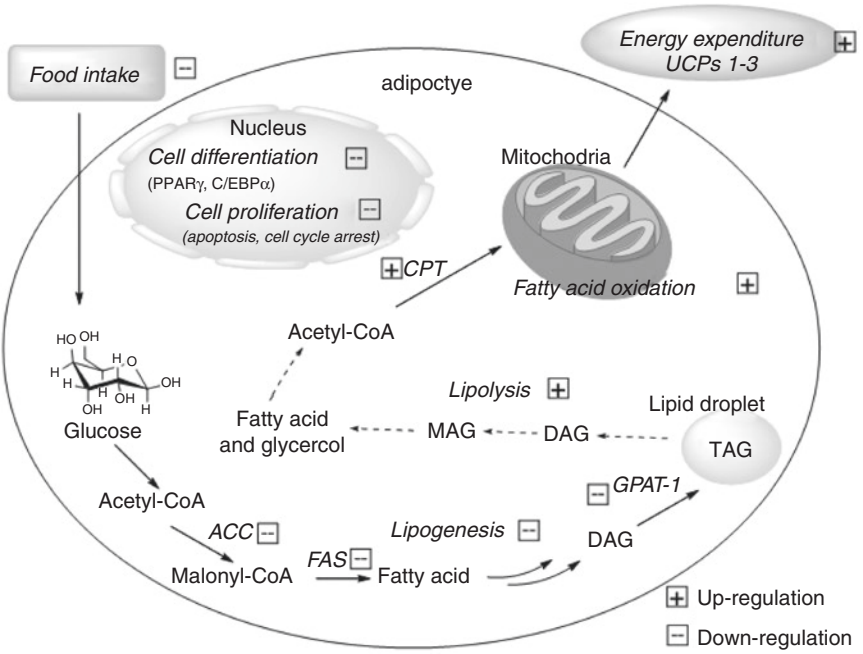

Fig. 13.17 The possible molecular mechanism of action of dietary polyphenols on adipocyte: (1) decreasing food intake; (2) inhibition of cell differentiation and proliferation; (3) inhibition of lipogenesis and adipogenesis; (4) promoting lipolysis; (5) stimulating fatty acid oxidation; (6) increasing energy expenditure. Abbreviations: MAG, monoglyceride; DAG, diglyceride

enhancer-binding protein α (C/EBPα). PPARγ and C/EBPα regulate the downstream adipogenic and lipogenic genes, including acetyl-coenzyme A carboxylase (ACC) that promotes fatty acid synthesis and inhibits fatty acid oxidation and the transcriptional factor sterol regulatory element-binding protein 1c (SREBP-1c), which enhances lipogenesis and adipogenesis (Wakil and Abu-Elheiga 2009). Furthermore, AMPK inhibits ACC via phosphorylation and prevents fatty acid synthesis and fat accumulation. ECCG inhibits lipogenesis and adipogenesis by activating AMPK and inhibiting ACC and SREBP-1c (Kim et al. 2010; Murase et al. 2009; Chan et al. 2011).

Hormone-sensitive lipase (HSL) is a key enzyme involved in hydrolysis of stored triacylglycerol to monoacylglycerol and free fatty acids. EGCG upregulates HSL level, stimulates lipolysis, and thus depletes fat accumulation and adipogenesis in 3T3-L1 adipocytes (Lee et al. 2009). Furthermore, EGCG promotes thermogenesis by upregulating uncoupling protein 2 (UCP2), a key protein regulating fat-supported thermogenesis (Lee and Kim 2009). Animal studies using high-fat-induced obesity in rat confirm EGCG's anti-obesity properties. In mice fed a high-fat diet (60% energy as fat), supplementation with EGCG reduces body weight and body fat, decreases plasma cholesterol and liver triglycerides levels, and inhibits lipid accumulation in hepatocytes (Bose et al. 2008). Epidemiological and clinical studies

suggest that consumption of green tea or catechins reduces body weight, waist and hip circumference, and total body fat. However, their effects on lipid profiles (total cholesterol, low-density lipoprotein cholesterol, free fatty acids, triacylglycerol) are inconsistent. Therefore, green tea and catechins reduce body weight by enhancing energy expenditures and fatty acid oxidation (Wang et al. 2014).

Curcumin and resveratrol have anti-obesity effects by inhibition of adipocyte proliferation and differentiation, promotion of fatty acid oxidation and lipolysis, as well as restraining lipogenesis and adipogenesis (Meydani and Hasan 2010, Wang et al. 2014). Curcumin and resveratrol also facilitate weight loss by interacting with distinct targets. Curcumin enhances fat acid β-oxidation in adipocytes by upregulating the expression of carnitine palmitoyltransferase-1 (CPT-1), a key enzyme involved in the transfer of acyl-CoA into the mitochondria for β-oxidation (Ejaz et al. 2009). It downregulates the expression of fatty acid synthase (FAS) and glycerol-3-phosphate acyltransferase-1 (GPAT-1), two key enzymes regulating fatty acid and triacylglycerol synthesis, respectively (Ejaz et al. 2009; Zhao et al. 2011). Resveratrol activates sirtuin 1 (SIRT1) that deacetylates forkhead box protein O1 (FOXO1) and consequently prevents transcription of PPARγ and lipid accumulation (Ahn et al. 2008). Animal studies support the roles of curcumin and resveratrol in the prevention and improvement of obesity and obesity-related disorders. However, convincing results in support of anti-obesity property of curcumin and/or resveratrol in human are still limited.

13.3.8 Osteoporosis Prevention

Osteoporosis is a chronic and progressive disease of multifactorial etiology characterized by decreases of bone mass and density, and hence producing weak bone strength that allow fractures to occur with minimal trauma (Marcus and Majumder 1996). Osteoporosis can be classified as three types: (1) primary type 1 osteoporosis that is a skeletal disorder in women after menopause and also named postmenopausal osteoporosis; (2) primary type 2 osteoporosis that is a skeletal disorder commonly occurs in men and women older than 75 and also named senile osteoporosis; and (3) secondary osteoporosis, which refers to bone loss caused by predisposing medical diseases or therapeutic agents. In primary type 1 osteoporosis, estrogen deficiency in postmenopausal women is a principal cause, and hormone replacement is the therapeutic strategy for preventing decreased bone mass and density. However, hormone replacement therapy can give rise to side effects and long-term risks. Being structurally similar to the estrogen nucleus (Fig. 13.18), isoflavones, an important group of phytoestrogen, display estrogen-like activity and can be regarded as an alternative approach for osteoporosis prevention at optimal dosages (Anderson and Garner 1997).

The isoflavone-type phytoestrogen, genistein, daidzein, and their glycosides, were evaluated as preventive therapies against osteoporosis using ovariectomized rat models (Ishida et al. 1998; Ishimi et al. 2000; Picherit et al. 2000;

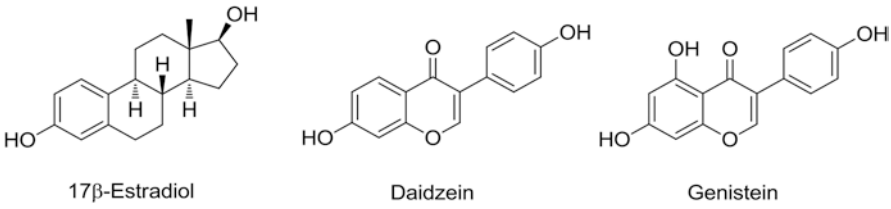

Fig. 13.18 Comparison of the molecular structures of typical isoflavone with estrogen 17β-estradiol

Nakajima et al. 2001). Supplementation with genistein, daidzein, or genistin prevents ovariectomy-induced pathological changes of bone mineral density. Oral administration of daidzein at doses of 10 mg/kg/day for 3 months inhibits the losses of bone mineral density in lumbar vertebrae, femur, and its metaphyseal and diaphyseal zones, whereas genistein at doses of 10 mg/kg/day only prevents the bone mineral density in the diaphyseal zone. Furthermore, daidzein, but not genistein inhibits ovariectomy caused atrophy of the uterus (Ishida et al. 1998). These data suggest that daidzein was more active than genistein and that these two phytochemicals have different mechanism of action (Picherit et al. 2000). Genistein exerts its protection against osteoporosis via stimulation of bone formation rather than on suppression of bone resorption (Fanti et al. 1998). A study in ovariectomized rats indicates that isoflavones prevent bone loss but does not reverse the established osteopenia (Picherit et al. 2001). Because soy is rich in these isoflavones, consumption of soybean and soy food improves bone mineral density in postmenopausal women. Soy-rich diets could be effective in reducing the risk of osteoporosis in postmenopausal women (Scheiber et al. 2001; Chiechi et al. 2002). Rutin is a glycoside of quercetin and occurs in fruits and fruit rinds, for example, in lemon, orange, apple, and berry. Addition of rutin to the diet of ovariectomized rats inhibits the ovariectomy-induced total and distal metaphyseal femoral mineral density (Horcajada Molteni et al. 2000).

13.4 Conclusions

Consumption of food and beverages rich in polyphenols plays a role in the prevention of NCDs, including cancer, diabetes, CVDs, neurodegenerative diseases, and obesity. Modulation of multiple cell signaling pathways can explain the antioxidant effects of dietary polyphenols. These mechanisms of action of polyphenols include modulation of Nrf2, MAPK, AP-1, NF-kB, and caspases, as well as COX and LOX. These actions can explain their diverse pharmacological functions. Polyphenols exert their effects on these pathways either separately or sequentially. Additionally, possible cross talk between these pathways is an important approach for prevention of pathological process.

In vitro and in vivo evidence supports a role for dietary polyphenols in the prevention of development and progression of NCDs associated with oxidative stress

and inflammation. However, prevention of human diseases by dietary polyphenols has yet to be demonstrated again in a convincing manner. Bioavailability of polyphenols is a critical factor that can impact their capacity to counteract ROS-induced oxidative damage in the human organs. An understanding of polyphenol absorption, distribution, metabolism, and excretion will facilitate the application of polyphenols as a preventive therapeutic strategy against human diseases.

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Chapter 14
Plant Flavonoids in Health, Prevention, and Treatment of Chronic Diseases

Aleksandra Kozłowska and Dorota Szostak-Węgierek

Abstract Noncommunicable diseases (NCDs), also known as chronic diseases, principally cardiovascular disease (CVDs), diabetes, neurodegenerative diseases, and cancer, continue to be a burden for health systems in both developed and developing countries. NCDs and their complications are responsible for an increased number of deaths and expensive medical costs. These and other factors make the prevention of NCDs an extremely high priority for clinical medicine and public health. Flavonoids are a group of bioactive compounds that are widely distributed in many plant-based foods and beverages. Epidemiological studies support the important role of high flavonoid intake in the prevention of a number of NCDs, including type 2 diabetes mellitus, CVDs, cataract, neurodegenerative disorders, and cancer. Moreover, in vitro and animal studies report important roles of flavonoids such as quercetin, kaempferol, naringenin, hesperitin, and luteolin, in the treatment of cardiovascular and neurodegenerative diseases and also cancer. Dietary flavonoids exert their cardioprotective effects by regulating blood pressure, protecting against oxidized low-density lipoprotein induced damage, and providing antiplatelet effects. Furthermore, they may protect the nervous system through the reduction of neuroinflammation and axonal damage. Anticancer actions of flavonoids depend on their ability to inactivate carcinogens and to enhance DNA repair processes. Flavonoids exert antioxidative and anti-inflammatory effects through their free radical scavenging properties; therefore, they play important roles in the prevention of metabolic

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abnormalities such as dyslipidemia, insulin resistance, hypertension, glucose intolerance, inflammation, and oxidative stress. There is a growing body of evidence that diets rich in flavonoids are beneficial for improved health and prevention of NCDs.

Keywords Antioxidants • Flavonoids • Dietary intake • Chronic disease prevention

14.1 Introduction

Noncommunicable diseases (NCDs), also known as chronic diseases, principally cardiovascular disease (CVDs), obesity, diabetes, cancer, and neurodegenerative diseases, represent the main disease burden in both developed and developing countries. NCDs and their complications are responsible for a significant number of deaths as well as expensive medical costs. Therefore, the prevention of NCDs is a high priority for public health and clinical medicine (Gan et al. 2015, Guirguis-Blake et al. 2016, Wallace et al. 2016). Unhealthy diets and lifestyle habits are among the main causes of NCDs. Plant-based diets can reduce the risk of NCDs. A diet rich in flavonoids plays an important role in prevention of NCDs and their complications (Pandey and Rizvi 2009, Grassi et al. 2010). Flavonoids exert antioxidant and anti-inflammatory effects at least in part through their free radical scavenging properties and enhancement of antioxidant enzymes in their actions in mitigating the onset and progression of various NCDs; therefore, they play important roles in prevention of metabolic abnormalities such as dyslipidemia, insulin resistance, hypertension, glucose intolerance, inflammation, and oxidative stress (Upadhyay and Dixit 2015).

Flavonoids are secondary metabolites of higher plants and over 10,000 structures have been identified to date. They are widely distributed pigments with important functions, such as protection against damage caused by UV radiation and visible light (Bornman et al. 1997, Tanaka et al. 2008). Flavonoids also act as visible signals to attract animals for pollination and seed dispersal. Flavonoids are primarily synthesized for the plants' own defense against oxidative stress. Importantly, they maintain antioxidant properties and contribute to the pharmaceutical and dietary attributes of plant foods, and as such the antioxidant properties of flavonoids are important for plant and human physiological functions (Pollastri and Tattini 2011, Csepregi et al. 2016). This chapter reviews the current knowledge of the protective role of flavonoids against NCDs by discussing the use of different types of flavonoids and their subclasses in the prevention and treatment of NCDs. Our search strategy used the standard clinical trial database (http://clinicaltrials.gov/) and the keyword "flavonoid." The search that was performed in April 2016 identified 112 clinical studies in which flavonoids were registered.

14.2 Flavonoids Subclasses, Sources, and Dietary Intake

Flavonoids constitute a class of polyphenols that may be further divided into the following six subclasses: anthocyanins, flavanols, flavanones, flavones, flavonols, and isoflavones (Table 14.1). Anthocyanins are present in many fruits, vegetables, flowers, grains, and other plant-derived foods and are responsible for generating their characteristic red, blue, and purple color (Wallace et al. 2016). These substances include anthocyanidins that are sugar-free aglycones and also anthocyanin glycosides. The six most commonly occurring anthocyanidins are cyaniding, delphinidin, malvidin, peonidin, pelargonidin, and petunidin. The main food sources of anthocyanins in the human diet are shown in Tables 14.2 and 14.3. Other flavonoids are flavanols that are present in tea, fruits, cocoa, and chocolate. They exist both as monomers (catechins) and polymers. Among catechins there are free forms, such as epicatechin, gallocatechin, and epigallocatechin (EGC), and also bound forms, such as epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). The content of flavanols in some foods is shown in Table 14.4, while Table 14.5 shows flavanone-rich products, where the content is abundant in oregano and citrus fruits. Hesperidin (HES) and its metabolite hesperetin and also naringenin are the main representatives of the flavanones subclass. Flavones, another flavonoid subclass, include substances such as sinensetin, isosinensetin, nobiletin, tangeretin, luteolin, apigenin, chrysin, and galangin, and the content in some foods is shown in Table 14.6. The most abundant sources of flavones are parsley and oregano. Flavonols are the most common flavonoids and are dispersed throughout the plant kingdom. The main dietary flavonols are kaempferol, quercetin, fisetin, isorhamnetin, and myricetin. The main dietary sources of flavonols such as capers, parsley, elderberry juice, and sorrel are shown in Table 14.7. The main representatives of isoflavones such as daidzein and genistein that are present primarily in soy foods are shown in Table 14.8.

Important dietary sources of flavonoids are vegetables, fruits, seeds, and some cereals, together with wine, tea, and certain spices (Table 14.1). The most abundant sources are berries, cowpeas, dock, kale, dark chocolate, parsley, oregano, capers,

Table 14.1 Subclasses of flavonoids; authors' selection based on Bhagwat et al. (2013) and Csepregi et al. (2016)

Subclass	Examples of compounds
Anthocyanins	Pelargonidin, cyanidin, delphinidin, peonidin, petunidin, malvidin
Flavanols	Epicatechin, catechin, epicatechin gallate (ECG), gallocatechin, epigallocatechin (EGC), epigallocatechin gallate (EGCG)
Flavanones	Naringenin, hesperetin
Flavones	Sinensetin, isosinensetin, nobiletin, tangeretin, luteolin, apigenin, chrysin, galangin
Flavonols	Kaempferol, quercetin, fisetin, isorhamnetin, and myricetin
Isoflavones	Daidzein, genistein

Table 14.2 Content of flavonoids in chosen foodstuffs (mg/100 g foodstuff); authors' selection based on Bhagwat et al. (2013)

Flavonoids (mg/100 g)					
Fruit		*Vegetables*		*Spices and herbs*	
Elderberries	518.13	Dock	102.20	Parsley, dried	4854.49
Chokeberry	368.66	Kale	92.98	Oregano, Mexican, dried	1545.79
Currants, black	167.47	Fennel	84.50	Capers, raw	493.03
Blueberries	158.51	Arugula	69.27	Parsley, fresh	233.16
Blackberries	137.66	Cabbage, red	64.34	Peppermint, fresh	60.48
Cranberries	113.58	Onions, red	44.87	Thyme, fresh	47.75
Currants, red	79.49	Radishes	26.52	*Nuts*	
Kumquats	79.26	Celery hearts, green	22.60	Pecans	34.01
Grapefruit	55.40	Artichokes	22.20	Pistachio	14.37
Lemons	53.38	Chives	21.67	Hazelnuts	11.96
Limes	48.60	Peppers, hot chili	21.17	Almonds	11.00
Grapes, red	48.35	Broad beans, cooked	20.63	*Beverages*	
Raspberries	47.58	Lettuce, red	19.40	Green tea, brewed	121.27
Oranges	43.49	Asparagus, cooked	15.16	Black tea, brewed	119.32
Cherries	40.00	Cress, fresh	14.00	Black currant juice	78.04
Strawberries	34.31	Chicory, green	11.79	White tea, brewed	74.60
Pears	21.53	Spinach	11.44	Oolong tea, brewed	52.37
Plums	14.23	Chard	10.43	Wine, table red	40.84
Apple	13.73	Endive	10.10	Orange juice	24.13
Bananas	13.69	Brussels sprouts, cooked	7.68	Pink grapefruit juice	17.97
Apricots	10.67	Peppers, green	6.98	Beer	3.34
Legumes		*Sweets*		*Cereals and grains*	
Cowpeas	277.41	Dark chocolate	108.60	Wheat, purple	25.85
Beans, black	28.00	Cocoa, dry powder	106.68	Buckwheat	15.38
Beans, kidney, red	10.87	Milk chocolate	15.04	Sorghum, red	8.43

and green and black tea. It is important to note that the presence of particular flavonoids in vegetables and fruits depends on the crop variety, location, and type of cultivation, as well as the specific plant morphological component. Differences in flavonoid contents between plant species are usually small, although in a few cases, very high amounts have been observed, e.g., in certain berries and tea prepared from leaves of the Quingmao tree (Bhagwat et al. 2013).

The consumption of flavonoids in a plant-based diet is usually several times higher than the consumption of other phytochemicals and vitamins, including ascorbic acid (vitamin C), α-tocopherol (vitamin E), or carotenoids (Lotito et al. 2011). There are several databases of flavonoid contents in foods, which can be used to estimate their intake in the human diet. The most commonly used are United States Department of Agriculture (USDA) databases and the online Phenol-Explorer database (www.phenol-explorer.eu). For that reason, a comparison of the results of

Table 14.3 Content of anthocyanins in chosen foodstuffs (mg/100 g foodstuff); authors' selection based on Bhagwat et al. (2013)

Anthocyanins (mg/100 g)					
Elderberry juice concentrate	411.40	Blackberries	90.64	Pecan nuts	25.02
Chokeberry	349.79	Red currants	75.02	Red table wine	23.18
Bilberries	285.21	Red cabbage	63.50	Black grapes	21.63
Chickpeas	262.49	Raspberries	40.63	Pears	12.18
Black currants	154.77	Red bilberries	40.15	Morello cherries	7.45
American bilberries	141.03	Strawberries	27.76	Hazel nuts	6.71
Cyanidin (mg/100 g)					
Elderberries	485.26	Cowpeas, black seeds	94.72	Raspberries	45.77
Elderberry juice concentrate	411.10	Bilberry	85.26	Cherries	32.57
Chokeberry	344.07	Red currants	65.54	Plums	17.93
Red cabbage	209.83	Black currants	62.46	Acerola	15.71
Radicchio	126.99	Acai berries	53.64	Sweet potato, cooked	10.60
Blackberries	99.95	Cranberries	46.43	Blueberries	8.46

Table 14.4 Content of flavanols in chosen foodstuffs (mg/100 g foodstuff); authors' selection based on Bhagwat et al. (2013)

Flavanols (mg/100 g)					
Green tea, brewed	116.15	Blackberries	42.50	Apples	9.17
Black tea, brewed	115.57	Cooked broad beans	20.63	Peaches	8.60
Dark chocolate	108.60	Pecan nuts	15.99	Apricots	8.41
Cocoa, dry powder	52.73	Red table wine	11.05	Apple juice	5.96
Epigallocatechin gallate (mg/100 g)					
Carob flour	109.46	Green tea, brewed	19.97	Pecan nuts	2.30
White tea, brewed	42.45	Black tea, brewed	9.36	Hazelnuts	1.06
Oolong tea, brewed	34.48	Fruit tea, brewed	4.15	Plums	0.4
Catechin (mg/100 g)					
Carob flour	50.75	Pecan nuts	7.24	Pistachio nuts	3.57
Dark chocolate	11.99	Red, table wine	7.14	Strawberries	3.11
Broad beans	8.16	Peaches	4.92	Plums	2.89

nutritional assessments obtained on the basis of the various data sources may differ. It should be emphasized that many surveys were performed in the 1990s and may be underestimates, as the database of flavonoid levels in foods was incomplete at that time. For proper interpretation of the results regarding assessment of dietary flavonoids intake, the year of release of specific databases should be taken into account.

Table 14.5 Content of flavanones in chosen foodstuffs (mg/100 g foodstuff); authors' selection based on Bhagwat et al. (2013)

Flavanones (mg/100 g)					
Dried Mexican oregano	412.13	Limes	46.40	Grapefruit juice	18.98
Grapefruit	54.50	Oranges	42.57	Artichokes	12.51
Lemons	49.81	Orange juice	18.99		
Hesperetin (mg/100 g)					
Limes	43.00	Oranges	27.25	Peppermint, fresh	10.16
Yuzu	28.73	Lemon juice	14.47	Pummelo	8.40
Lemons	27.90	Orange juice	11.95	Tangerines	7.94
Naringenin (mg/100 g)					
Dried Mexican oregano	372.00	Yuzu	24.82	Oranges	15.32
Grapefruit	53.00	Pummelo	24.72	Tangerines	10.02
Rosemary, fresh	24.86	Pink grapefruit juice	17.19	Limes	3.20

Table 14.6 Content of flavones in chosen foodstuffs (mg/100 g foodstuff); authors' selection based on Bhagwat et al. (2013)

Flavones (mg/100 g)					
Dried parsley	4523.25	Artichokes	9.69	Chicory	2.85
Dried oregano	1046.46	Green pepper	4.71	Lemons	1.90
Fresh parsley	216.15	Celeriac	3.90	Red grapes	1.30
Apigenin (mg/100 g)					
Dried parsley	4503.50	Celery hearts, green	19.10	Rutabagas, raw	3.85
Fresh parsley	215.46	Wild carrots	12.6	Oregano, fresh	2.57
Vine spinach	62.20	Artichokes, raw	7.48	Sorghum, grain, red	2.54
Kumquats, raw	21.87	Peppermint, fresh	5.39	Peppers, hot chili, green, raw	1.40
Luteolin (mg/100 g)					
Thyme, fresh	45.25	Sage, fresh	16.70	Peppers, hot chili, green, raw	3.87
Radicchio, raw	37.98	Peppermint, fresh	12.66	Chicory	2.08
Celery, Chinese, raw	34.87	Peppers, sweet, green, raw	4.71	Lemons, raw	1.90
Wild carrots	34.1	Sorghum, grain, red	3.93	Kohlrabi	1.30

Recent evidence suggests that the total daily intake of flavonoids varies among nations and cultures. The mean daily total flavonoid intake in US adults was estimated by Kim and coworkers (NHANES food consumption data 2007–2010, $n = 9801$) and is equal to 200.1 ± 8.9 mg/day (Kim et al. 2016a). The mean total intake of flavonoids among European adults (more than 30,000 participants) is 428 mg/day with the highest intake in the Central Region (506 mg/day), followed by the Northern Region (348 mg/day), and the Southern Region (301 mg/day)

Table 14.7 Content of flavonols in chosen foodstuffs (mg/100 g foodstuff); authors' selection based on Bhagwat et al. (2013)

Flavonols (mg/100 g)					
Fresh capers	493.03	Goji berries	31.20	Chicory	8.94
Dried parsley	331.24	Fresh cranberries	21.59	Buckwheat	7.09
Elderberry juice concentrate	108.16	Cooked asparagus	15.16	Dried and sweetened cranberries	6.91
Sorrel	102.20	Blackcurrants	11.53	Fresh figs	5.47
Rocket lettuce	69.27	American bilberries	10.59	Cooked Brussels sprouts	5.24
Red onions	38.34	Morello cherries	9.41	Apples	3.40
Quercetin (mg/100 g)					
Fresh capers	233.84	Fennel, leaves, raw	48.80	Chia, seeds	18.42
Lovage, leaves, raw	170.0	Red onions	39.21	Cranberries, raw	14.84
Radish, leaves, raw	70.37	Elderberries, raw	26.77	Asparagus	13.98
Coriander, leaves, raw	52.90	Kale, raw	22.58	Goji berry, dried	13.60
Kaempferol (mg/100 g)					
Fresh capers	259.19	Ginger	33.60	Chard, red leaf	9.20
Saffron	205.48	Cress, raw	13.00	Collards, raw	8.74
Kale, raw	46.80	Endive, raw	10.10	Broccoli	7.84
Arugula, raw	34.89	Chives, raw	10.00	Spinach, raw	6.38

Table 14.8 Content of isoflavones in chosen foodstuffs (mg/100 g foodstuff); authors' selection based on USDA (2007)

Isoflavones (mg/100 g)					
Soy flour	166.66	Soybeans, raw	48.95	Clover, red	21.0
Soybeans mature seeds (Europe)	103.56	Miso	41.45	Soybeans, cooked	17.92
Natto	82.29	Soy yoghurt	33.17	Tofu	13.75
Tempeh	60.61	Tofu, different types	28.91	Pistachio nuts	3.63
Genistein (mg/100 g)					
Soybeans mature seeds (Europe)	39.78	Soybean chips	27.45	Soy yoghurt	16.59
Natto	37.66	Miso	23.24	Tofu	15.69
Tempeh	36.15	Soy paste	17.79	Soy cheese, unspecified	11.14
Daidzein (mg/100 g)					
Soybeans mature seeds (Europe)	45.44	Tempeh	22.66	Soy yoghurt	13.77
Natto	33.22	Soy paste	19.71	Tofu	11.64
Soybean chips	26.71	Miso	16.43	Clover, red	11.0

(Bhaswant et al. 2015). The mean total flavonoid intake in the Polish population (6666 participants of the Polish National Multicenter Health Survey), which was calculated with the use of the Phenol-Explorer database, is 403.5 mg/day. In contrast, when calculated on the basis of the USDA databases, this is equal to 525 mg/day (Witkowska et al. 2015). These results contrast with our own findings where the average daily flavonoid consumption among Polish students is 801 mg (Kozłowska et al. 2015). In turn, an investigation performed in a subsample of older Australians ($n = 79$) reported a mean intake of flavonoids of 683 mg/day, of which flavan-3-ols contributed 92%, followed by flavonols (4%), flavanones (3%), and flavones (<1%) (Kent et al. 2015). Importantly, the daily intake of total flavonoids varies widely depending on food choices, group of population, and age.

Besides their natural sources, there is also a wide choice of flavonoid supplements available on the market. Providers of these preparations recommend daily flavonoid doses that are higher than those normally achieved from a flavonoid-rich diet. Thus, there is a concern about potential adverse effects, such as decreases in trace elements bioavailability, antithyroid and goitrogenic actions, and flavonoid-drug interactions (Chandra and De 2010, de Souza dos Santos et al. 2011, Egert and Rimbach 2011, Giuliani et al. 2014, Ma et al. 2010). A growing body of evidence indicates that consumption of dietary flavonoids is safe and that dietary flavonoids and their condensed sources play beneficial roles in disease prevention; however, further clinical and epidemiological trials are urgently needed.

14.3 Flavonoids and the Related Health Benefits

The increasing global prevalence of NCDs has become a serious public health challenge of the twenty-first century (Chen et al. 2012). Prevention of these diseases is a priority, and a diet rich in flavonoids may represent a cost-effective and easily implemented preventive strategy (Kozłowska and Szostak-Węgierek 2014). Epidemiological and randomized controlled trials suggest a protective effect of foods and dietary supplements rich in flavonoids against NCDs, including CVDs, type 2 diabetes mellitus (T2DM), obesity, cancer, Alzheimer's disease (AD), and Parkinson's disease (PD). In this subsection, we tried to summarize results of epidemiological trials and show the main mechanisms of flavonoid action.

14.3.1 Flavonoids in Cardiovascular Disease

A diet rich in fruits and vegetables has long been linked to a reduced risk of CVDs, and an association was reported in numerous studies. The risk of CVDs was reduced by 12% for every increment of 477 g/day of total fruit and vegetable con-

sumption, by 16% for that of 300 g/day of fruit intake, and by 18% for 400 g/day of vegetable consumption (Gan et al. 2015). Similarly, in the European Prospective Investigation into Cancer and Nutrition study, participants who consumed at least eight portions (80 g each) of fruits and vegetables a day had a 22% lower risk of fatal ischemic heart disease (IHD) in comparison with those who consumed less than three portions a day (Crowe et al. 2011). In a subsequent study, a linear dose-response relationship indicated that the risk of stroke decreased by 32% and 11% for every daily 200 g increment in fruit and vegetable consumption respectively (Hu et al. 2014).

Epidemiological evidence supports the recommendation for daily consumption of at least five servings of fruits and vegetables. This group of foods provides not only a number of important nutrients, such as fiber, folate, antioxidant vitamins, and potassium, but also an abundance of bioactive compounds among which flavonoids may be crucial in the prevention of CVDs (Lai et al. 2015, Macready et al. 2014). However, epidemiological studies that unequivocally demonstrate the relation between high flavonoid intake and the reduced CVDs risk are scarce. Nevertheless, results of a meta-analysis of 15 prospective studies suggest such an association (Jiang et al. 2015). The pooled results show that the highest flavonoids intake versus the low intake is related to a reduced risk of coronary heart disease (CHD). The authors also observed a nonsignificant inverse association, with a 5% decreased risk of CHD for every 20 mg/day increase in flavonoids intake (Jiang et al. 2015). However, while many studies demonstrate inverse associations between flavonoid intake and CVD incidence, the Framingham Offspring Cohort failed to support such observations (Jacques et al. 2015). The results show that there is no association between flavonoid intake and CHD incidence after multivariable adjustment. This finding, based on long-term follow-up of middle-aged and elderly populations, suggests that the relationship between flavonoid intake and CVD risk is not clear and requires further investigation.

14.3.2 Cardioprotective Action of Flavonoids

Flavonoids exert their beneficial effect on cardiovascular system through various mechanisms that are summarized in Fig. 14.1. While the direct mechanisms are not well understood, it appears that the actions of flavonoids are multifaceted and depend on parallel processes. Among the main pathways are antioxidant and anti-inflammatory activities, regulation of blood pressure, decreases in cholesterol levels, antiplatelet effects, and protection of low-density lipoprotein (LDL, the major atherogenic lipoprotein), from oxidative modification. Flavonoids can attenuate inflammatory processes through several mechanisms, including inactivation of nitric oxide ($NO^{\bullet}$), scavenging of reactive oxygen species (ROS), and also inhibition of the influx of leukocytes into sites of inflammation (Majewska-Wierzbicka and Czeczot 2012). A meta-analysis involving 215 participants from eight clinical trials

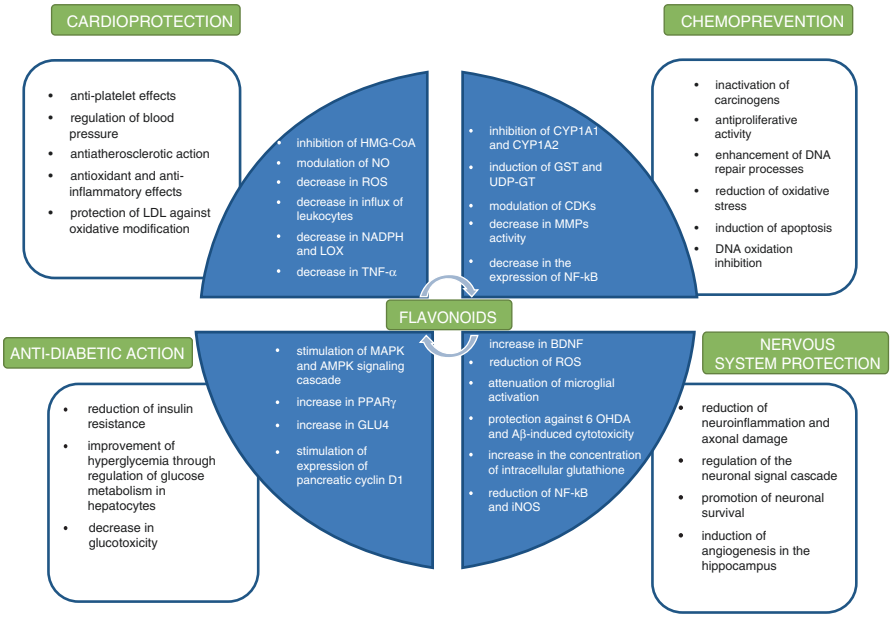

Fig. 14.1 Flavonoids and their effects on health

reports that consumption of flavanol-rich cocoa reduces plasma LDL cholesterol concentrations (Jia et al. 2010). Moreover, flavonoids modulate inflammation of the vascular endothelium and improve vascular function (Habauzit and Morand 2012). Furthermore, flavonoids decrease activities of the ROS-producing enzymes, xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and lipoxygenase (LOX) (Magalingam et al. 2015, Majewska-Wierzbicka and Czeczot 2012). Flavonoids stimulate 5′ adenosine monophosphate-activated protein kinase (AMPK) that leads to inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol synthesis. Consequently, increased AMPK activity reduces cholesterol synthesis serum levels (Wallace et al. 2016). Inactivation of proinflammatory enzymes, such as LOX and cyclooxygenase (COX), by flavonoids reduces the synthesis of thromboxane and leukotrienes and thus leads to the decreased in vasoconstriction (Borghi and Cicero 2016, Hugel et al. 2016). A flavonoid-rich diet may improve microvascular reactivity and inflammatory status in men at risk of CVDs. After 6 weeks of daily intake of additional two portions of high-flavonoid fruits and vegetables, the males under study exhibited increased endothelium-dependent microvascular reactivity. Moreover, after 12 weeks of daily intake of an additional four portions of high-flavonoid fruits and vegetables, they exhibited reduced plasma concentration of C-reactive protein, E-selectin, and vascular cell adhesion molecule (Macready et al. 2014). The antiplatelet activity of flavonoids is associated with $NO^{\bullet}$ formation in the vascular endothelium and simultaneous inhibition of the synthesis of 12-hydroxyeicosatetraenoic acid (12-HETE) a compound that impairs endothelial function (Liang et al. 2015a, Vaiyapuri et al. 2015).

14.3.3 Flavonoids in Type 2 Diabetes Mellitus and Maintenance of Body Weight

Epidemiological studies and meta-analyses suggested an inverse relationship between the consumption of flavonoid-rich diet and development of T2DM. The study by Liu et al. shows that an increased in flavonoid intake of 500 mg/day is associated with a significant T2DM risk reduction of 5%. In the subgroup analyses, the observed beneficial effects were reported in the US population, wherein the mean age was above 40 years, and in studies that lasted for more than 20 years (Liu et al. 2014). Evidence from other cohort studies and also randomized trials suggest that consumption of dietary flavonoids from particular subclasses had a better inverse relationship with T2DM than with total flavonoid intake. This particularly applies to flavonols, flavanols, and anthocyanins (Jacques et al. 2013, Wedick et al. 2012, Zamora-Ros et al. 2014). Thus, it seems that high flavonoid intake has an important role in T2DM prevention. It is well recognized that normal body mass maintenance is important in T2DM prevention and control. A US cohort study reports that high intake of foods rich in flavonoids favors weight maintenance in adulthood (Bertoia et al. 2016). This observation is in accordance with the study where higher dietary flavone, flavonol, and catechin intakes are associated with non-significant increases in body mass index over the 14 years of observation in women (Hughes et al. 2008). Thus, it is possible that a high flavonoid intake may decrease diabetes risk by maintaining body weight.

14.3.4 Antidiabetic Action of Flavonoids

Flavonoids can produce beneficial effects in diabetes by many parallel mechanisms (Fig. 14.1) as they interact with various metabolic and signaling pathways in pancreatic β-cells, liver, adipose tissue, and skeletal muscles. Flavonoids enhance the uptake of glucose by peripheral tissues, such as the skeletal muscle and white adipose tissues, through translocation of glucose transporter type 4 (GLUT4) vesicles to the plasma membrane. They influence β-cell mass and function, as well as energy metabolism and insulin sensitivity in peripheral tissues, while also stimulating AMPK and other kinases such as extracellular signal-regulated protein kinases 1 and 2 (ERK1/ERK2) and p38 mitogen-activated protein kinase (p38MAPK) which are essential for the maximal stimulation of glucose uptake in response to insulin (Babu et al. 2013).

14.3.5 Flavonoids in Cancer Prevention

Consumption of dietary flavonoids can reduce the risk of ovarian (Hua et al. 2016), breast (Chen et al. 2014a, Hui et al. 2013), and lung (Cutler et al. 2008, Tang et al. 2009, Woo and Kim 2013) cancers. Regular flavonoid consumption reduces the risk of lung cancer, particularly in women who had stopped smoking, but there is no

evidence of flavonoid on the risk of other cancers (Cutler et al. 2008). A meta-analysis of 35 studies (988,082 subjects and 8161 cases) indicates that consumption of total dietary flavonoids and most of the flavonoid subclasses was inversely associated with smoking-related cancer risk. In the subgroup analyses by cancer site, the total dietary flavonoid intake is inversely associated with aerodigestive tract cancer risk and marginally with lung cancer risk (Woo and Kim 2013). A meta-analysis of 12 studies indicates a reduced risk of breast cancer in postmenopausal women who consumed large amounts of flavonoids such as flavonols and flavones (Hui et al. 2013). The epidemiological data presented above concerns only prevention and that the results of the studies are not always unambiguous and applies only to some types of tumors.

14.3.6 Flavonoids in Cancer: Mechanisms of Action

Flavonoid affects many stages of carcinogenesis, such as initiation, promotion, and progression (Fig. 14.1). This includes, inactivation of carcinogens, inhibition of cell proliferation, enhancement of DNA repair processes, and reduction of oxidative stress. In the progression phase, flavonoids inhibit angiogenesis and induce apoptosis and cytotoxic or cytostatic actions against cancer cells. One of the most important mechanisms by which flavonoids exert their effects is through their interactions with phase I metabolizing enzymes that are responsible for metabolism of various endogenous or exogenous substrates, including activation of carcinogens. Flavonoids inhibit some cytochrome P450 isozymes, such as CYP1A1 and CYP1A2, and thus protect against cellular damage by carcinogens. Another mechanism of their action is related to mutagen detoxification through induction of the phase II enzymes, such as glutathione S-transferase (GST) and UDP-glucuronyltransferase (UDP-GT), which detoxify and eliminate carcinogens from the body (Chahar et al. 2011). The anticancer effects of flavonoids can also be explained by cell cycle inhibition. Cyclin-dependent kinases (CDKs), which are activated by mitogenic signals within the cell, are key regulators of cell cycle progression. Alteration and deregulation of CDK activity are pathogenic hallmarks of cancer. Various types of cancers are associated with hyperactivation of CDKs due to mutations of CDK genes or CDK inhibitor genes. For this reason, much research is focused on substances that can inhibit or modulate CDKs. These actions can be exhibited by flavonoids, such as genistein, quercetin, daidzein, luteolin, kaempferol, apigenin, and epigallocatechin (Kozłowska and Szostak-Węgierek 2014).

14.3.7 Flavonoids in Cognitive Functions

In the PAQUID study (Personnes Age'es QUID), dietary flavonoid consumption by the elderly supported their cognitive functions. Participants whose flavonoid intake was in the two highest quartiles had better cognitive function after 10 years of observation than those who consumed less, demonstrating that regular consumption of dietary flavonoids can maintain cognitive function during aging

(Letenneur et al. 2007). These results are consistent with the study performed by Samieri et al. where high flavonoid intake during midlife helped to maintain good health and well-being during aging among women (Samieri et al. 2014). In this large prospective study (13,818 women from the Nurses' Health Study), higher intake of several flavonoid subclasses (i.e., flavonols, flavones, flavanones, flavan-3-ol polymers, and anthocyanins), as well as total flavonoids in the age-adjusted analyses, was associated with better odds of healthy aging. Healthy aging was defined as survival to more than 70 years with maintenance of four health domains (no major chronic diseases or major impairments in cognitive or physical function or mental health).

In the analysis of each component of healthy aging, flavonoid subclasses such as flavones and flavanones are positively associated with two of four domains in the authors' definition of healthy aging, although associations are weaker than for overall healthy aging. For example, compared with women in the lowest quintile, women in the highest quintile of flavone and flavanone intakes have 32% and 24% better odds of no mental health limitations, respectively, and 23% and 15% better odds of no physical function limitations (Samieri et al. 2014). Some studies focus on the Mediterranean diet (MD) that is rich in flavonoids from vegetables, legumes, fruits, cereals, spices, and wine. Adherence to MD is associated with lower risk of developing both AD and mild cognitive impairment (MCI) (Gardener et al. 2012, Sofi et al. 2010 Yusufov et al. 2016). Furthermore, in the prospective Australian Imaging Biomarkers and Lifestyle Study of Ageing (AIBL), the dietary pattern analysis reveals that both MCI and AD patients have lower adherence to the MD compared with healthy control participants. A comprehensive meta-analysis and systematic review suggests that higher adherence to the MD is associated with a reduced risk of developing MCI and AD and a reduced risk of progressing from MCI to AD (Singh et al. 2014).

14.3.8 Effects of Flavonoids on the Nervous System

Important pathological mechanisms of neurodegenerative diseases, including AD, are increased oxidative stress and inflammation (Calsolaro and Edison 2016, Tramutola et al. 2016, Magalingam et al. 2015). Dietary flavonoids counteract oxidative stress-induced neuronal death (Solanki et al. 2015). The beneficial effects of flavonoids, consumed both with foodstuffs and flavonoid-enriched extracts, appear to be also related to other processes, such as regulation of the neuronal signal cascade that results in the inhibition of cell apoptosis caused by the neurotoxic substances, amyloid β (Aβ), and 6-hydroxydopamine (6-OHDA). Furthermore, flavonoids can also increase levels of brain-derived neurotrophic factor (BDNF), which promotes neuronal survival and differentiation. Flavonoids can exert beneficial effects on the peripheral and central nervous systems by improvement of blood flow, as they induce angiogenesis and growth of new nerve cells in the hippocampus, which are important for maintenance of neuronal and cognitive brain functions (Solanki et al. 2015) (Fig. 14.1).

14.4 Effects of Flavonoid Subclasses on Health, Prevention, and Treatment of Chronic Diseases

Much current research focuses on the efficacy of flavonoids in the prevention and treatment of hyperlipidemia, hypertension, postprandial hyperglycemia, platelet aggregation, cancer, and eye cataracts, as well as in the protection against neurodegenerative diseases. In order to acquaint the reader with many effects of some flavonoids in health and NCDs, we next describe results of some recent studies.

14.4.1 Anthocyanins

High intakes of anthocyanins (320 mg twice daily/4 weeks) do not reduce blood pressure (BP) in men ($n = 31$) with screening BP > 140/90 mm Hg (Hassellund et al. 2012). In a randomized, double-blind, placebo-controlled clinical trial, which included 50 hyperlipidemic adults, intake of anthocyanins (90 mg of anthocyanins from *Vaccinium arctostaphylos* extract/day/4 weeks) reduced total serum cholesterol levels by an average of 34.44 mg/dL, LDL-C by 11.44 mg/dL, TG by 69.64 mg/dL, and malondialdehyde (MDA) (a marker of lipid peroxidation) by 0.09 μmol/L but did not have any effect on high-density lipoprotein cholesterol (HDL-C) and high-sensitivity C-reactive protein (hs-CRP, a marker of inflammation) (Soltani et al. 2014). Another randomized, double-blind, placebo-controlled clinical trial ($n = 80$) reported that 2 months supplementation with extract from fruit rich in anthocyanins (whortleberry fruit, 350 mg capsule every 8 h) reduced serum levels of total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) by 27.6%, 19.2%, and 26.3%, respectively, and increased that of HDL-C by 37.5% (Kianbakht et al. 2014). These findings suggest that treatment with anthocyanin improves lipid profile in hyperlipidemic patients. Moreover, Zhu et al. (2015) demonstrate that supplementation with anthocyanins (320 mg/day/24 weeks) decreases proinflammatory markers in hypercholesterolemic subjects. Anthocyanin intake decreases serum levels of hs-CRP (−21.6% vs. −2.5%), soluble vascular cell adhesion molecule 1 (sVCAM-1) (−12.3% vs. −0.4%), and plasma interleukin 1 beta (IL-1β, −12.8% vs. −1.3%) compared to the placebo group (Zhu et al. 2013).

Anthocyanins represent promising substances for treatment of retinal and eye diseases (Chen et al. 2014b, Mok et al. 2014, Wang et al. 2015). Vascular endothelial growth factor (VEGF) is a key player in choroidal neovascularization. In the retina, VEGF is derived from retinal pigment epithelial (RPE) cells. In a study using cultured human RPE cells, cyanidin reduced the rate of RPE cell necrosis and decreased the secretion of VEGF without reducing cell viability (Chen et al. 2014b). Furthermore, cyanidin-3-glucoside (Cy-3-glu) isolated from three berries (blueberry, blackberry, and strawberry) has multiple antioxidant, anti-angiogenic, and anti-aging effects in human RPE cells. Cy-3-glu causes the largest reduction in

intracellular ROS level among the four anthocyanins tested (Cy-3-glu, pelargonidin-3-glucoside, delphinidin-3-glucoside, and malvidin-3-glucoside) under visible light irradiation. Moreover, Cy-3-glu reduces the VEGF level (Wang et al. 2015).

Anthocyanins can also protect against cataract formation. It is likely that in the pathogenesis of this cataract formation, apoptosis of the lens epithelial cells plays an important role and that oxidative stress may be a major cause of lens opacity. Anthocyanin from black soybean protects a human lens epithelial cell line (HLE-B3) from oxidative stress. This substance reduces HLE-B3 cell death under oxidative stress condition (Mok et al. 2014). Collectively, anthocyanins are promising agents in prevention and treatment of hyperlipidemia and some eye diseases.

14.4.2 Flavanols

Consumption of green tea, which is an important source of flavanols, is inversely associated with the risk for CVDs and also cardiovascular and overall mortality (de Koning Gans et al. 2010, Kokubo et al. 2013). An important mechanism of cardioprotection is vasodilation. EGCG-induced vasodilation strongly depends on functional $NO^{\bullet}$ synthase (NOS) in endothelial cells and subsequent stimulation of $NO^{\bullet}$ production in vessels. Low concentrations of EGCG (5–15 μM) cause $NO^{\bullet}$-dependent vasorelaxation in aortic rings of wild-type mice, (Lorenz et al. 2015). Black tea consumption (15 mg/kg/day) reduces hypertension-associated endothelial dysfunction in rats. It is likely that EGCG alleviates endoplasmic reticulum stress by downregulating homocysteine metabolic enzymes and attenuating ROS production (San Cheang et al. 2015).

Green tea catechins improve energy metabolism at rest and during exercise, both after short- or long-term green tea extract intake (Hodgson et al. 2013a, b). In a small randomized, double-blind, placebo controlled, crossover trial, eighteen patients with relapsing-remitting multiple sclerosis (MS) received EGCG (600 mg/day) or placebo over 12 weeks (4-week washout period in between). This supplementation improves muscle metabolism during moderate exercise to a greater extent in men than in women, possibly because of sex-specific effects on autonomic and endocrine control. Adipose tissue and skeletal muscle perfusion are lower in men but higher in women receiving EGCG, while fasting adipose tissue lactate concentrations are about two-fold higher in men receiving placebo, indicating an increased lipolytic activity, but normalized with EGCG. This study shows favorable effects of a combination of EGCG with physical activity in men with MS (Mahler et al. 2015). EGCG from green tea is an effective agent against bacterial infection, as evidenced by a decrease in bacterial translocation across porcine intestinal epithelial IPEC-J2 cells. EGCG appears also to enhance the epithelial immunological barrier by inducing secretion of the antimicrobial peptides, porcine beta-defensin 1 and 2 (pBD-1, pBD-2). This produces a protective effect against bacterial translocation by enhancing the immunological barrier, particularly in colitis ulcerosa and Crohn's disease (Wan et al. 2016).

Numerous studies have reported antiviral effects of flavanols, especially of EGCG, in animal and cell culture studies (Cantatore et al. 2013, de Oliveira et al. 2013, Liang et al. 2015b, Muller and Downard 2015, Zhong et al. 2015). Oral infection with herpes simplex virus type 1 (HSV-1) or genital infection with HSV type 2 (HSV-2) represents the most common infectious diseases in humans. Cantatore et al. evaluated the effects of black tea extract containing primarily dimer forms of different catechins called the aflavins on adenocarcinomic human alveolar basal epithelial cells and Vero-cultured cells (one of the more commonly used mammalian cell lines in microbiology), where black tea extract treatment reduced or blocked production of infectious HSV-1 virions. These effects are mediated through inhibition of the infectivity of the virus by interference with the attachment, penetration, and viral DNA replication of HSV-1 particles (Cantatore et al. 2013). In addition, modification of green tea flavanol EGCG with palmitate increases the effectiveness of EGCG as a potent inhibitor of HSV-1 (de Oliveira et al. 2013). Flavanols also influence autophagy, which plays a crucial role in the regulation of viral replication. Enhancement of autophagy is therefore a potential therapeutic strategy for viral infection (Li et al. 2011). In cultured hepatoma HepG2 cells, EGCG treatment increases lysosomal acidification in autophagy, which inhibited HBV replication (Zhong et al. 2015). Flavanols contribute to cardiovascular protection and probably also to prevention of viral and bacterial infections, with some improvement of muscle metabolism.

14.4.3 Flavanones

Flavonoids belonging to the flavanone subclass, such as HES and its aglycone form hesperetin, can contribute to cardiovascular health benefits, by reducing blood cholesterol level, inflammation, and blood pressure. Hypocholesterolemic actions of HES result from inhibition of human HMG-CoA reductase activity (Lee et al. 2012). This enzyme plays a key role in the synthesis of cholesterol in humans and thereby influences plasma cholesterol levels. Inhibition of its activity lowers intracellular cholesterol concentrations and results in increased expression of LDL receptors. This in turn raises cellular lipoprotein uptake and removal of cholesterol from the circulation. In a randomized, double-blind study, performed in subjects with metabolic syndrome ($n = 24$), oral administration of 500 mg of HES daily over 3 weeks stimulated endothelial $NO^{\bullet}$ formation and decreased the activity of proinflammatory cytokines (Rizza et al. 2011). In spontaneously hypertensive rats, HES (diet content of 0.1%) and glucosyl hesperidin (GHES) (diet content of 0.1%) prevented hypertension and suppressed the mRNA expression of NADPH oxidase subunits and thromboxane A2 synthase (Yamamoto et al. 2013).

Hesperetin and naringenin from bioconverted *Citrus unshiu* (a Mandarin citrus variety) peel extracts altered the adipogenic activity in 3T3-L1 preadipocytes culture cells by inhibition of lipid accumulation and suppression of adipocyte-specific transcription factors, including CCAAT-enhancer-binding protein alpha (C/EBPα),

peroxisome proliferator-activated receptor gamma (PPARγ), and sterol regulatory element-binding protein 1c (SREBP1c) (Lim et al. 2015). Naringenin supplementation may be beneficial for the treatment of metabolic syndrome, including obesity, diabetes, hypertension, and hyperlipidemia (Alam et al. 2014, Orhan et al. 2015). Moreover, in streptozotocin-induced diabetic rats fed a high-fat diet, naringenin improved postprandial hyperglycemia. Oral intake of naringenin (25 mg/kg body) inhibits intestinal α-glucosidase activity and thereby delays the absorption of carbohydrates and lowers postprandial blood glucose levels in the rat model of T2DM (Priscilla et al. 2014). Naringenin also influences other mechanisms that exacerbate metabolic complications of obesity. One of such pathway is macrophage infiltration into adipose tissue to induce chronic inflammation and leading to obesity-related diseases, such as type 2 diabetes and atherosclerosis (Suganami and Ogawa 2010). Naringenin suppresses macrophage infiltration into the adipose tissue in an early phase of high-fat diet-induced obesity in mice (Yoshida et al. 2014). In obese ovariectomized mice (an animal model of menopause), naringenin treatment reversed diet-induced metabolic disturbances (Ke et al. 2015, 2016). Dietary supplementation with naringenin (3% in diet) suppresses weight gain, lowers hyperglycemia, decreases intra-abdominal adiposity, and attenuates muscle loss (Ke et al. 2016). Moreover, levels of plasma leptin and leptin mRNA in adipose depots are also decreased in mice fed a naringenin diet. Monocyte chemoattractant protein-1 (MCP1) and interleukin 6 (IL-6) mRNA expression levels are significantly lower in perigonadal adipose tissue of naringenin-supplemented mice (Ke et al. 2015).

There are only a few human studies on the role of flavanones in diabetes prevention. However, high consumption of flavanones is associated with a reduced risk of diabetes. It is estimated that there are 314 new cases of diabetes over a mean of 5.51 years of follow-up (18,900 person-years), but after multivariable adjustment, there is a 31% reduction in new-onset diabetes in the lowest tertile of flavanone intake (Tresserra-Rimbau et al. 2016). A pilot study of childhood obesity-related traits in Mexican-American children detected 14 metabolites with nominal or significant differences in concentration between normal-weight, overweight, and obese children (Farook et al. 2015). One of these is naringenin, which remained significant even after adjusting for multiple variables. Overweight and obese children have decreased levels of naringenin compared with normal-weight children. The authors suggested that the normal-weight children might consume more dietary naringenin sources, such as grapefruit, orange, and tomato skin. Among obese children, the levels of naringenin were only half the levels seen in normal-weight children. The study also reported a negative association between naringenin levels and systolic blood pressure, diastolic blood pressure, homeostatic model assessment of insulin resistance (HOMA-IR), body mass index, waist circumference, and triglycerides and a positive correlation with HDL-C. The authors emphasized that risk biomarkers, available early in life, would enable early-life screening and interventions in high-risk children to prevent the development of serious health problems later in life (Farook et al. 2015).

HES and its derivative induce apoptotic cell death in gastric, colon, breast, lung, and liver cancers, which was mediated by nuclear factor-kappa B (NF-κB)

(Bartoszewski et al. 2014, Bodduluru et al. 2015, Devi et al. 2015, Wang et al. 2016). HES has an antiproliferative action against benzo(a)pyrene (B[a]P)-induced lung cancer in the Swiss albino mouse model. Oral administration of HES (50 mg/kg body weight) suppresses B[a]P-induced lung carcinoma and its associated preneoplastic lesions by alleviating lipid peroxidation, modulating antioxidant activities and reducing the expression of NF-κB, and proliferating cell nuclear antigen (PCNA) and CYP1A1 (B[a]P metabolic activator) (Bodduluru et al. 2015). Future studies should focus on methods of increasing the bioavailability and absorption of HES and also on detailed molecular mechanisms of anticancer effects of HES, determination of the most effective doses for future clinical trials on HES, and finally evaluation of anticancer effects of HES in patients with cancer (Devi et al. 2015). Altogether, there is evidence supporting a cardioprotective and anticancer action of flavanones. They can also ameliorate the metabolic complications of obesity.

14.4.4 Flavones

The major dietary flavones are apigenin and luteolin. It appears that apigenin and also chrysin have important roles in the attenuation of sugar-induced cataractogenesis. Aldose reductase (AR) is a key enzyme of the polyol pathway and is responsible for the reduction of glucose and galactose. Less than 3% of glucose is metabolized through polyol pathway under normoglycemic condition. However, in the case of hyperglycemia, more than 30% of glucose enters the polyol pathway, which leads to excess accumulation of sorbitol in the lens; this in turn results in several adverse pathological changes which, ultimately accelerates the process of cataractogenesis and other diabetic complications. The mechanism of the anticataract actions of apigenin and chrysin includes inhibition of AR as shown in sugar-induced lens cataract model studies (Bhatnagar and Srivastava 1992, Patil et al. 2016).

The animal model of PD is based on striatal damage by the neurotoxic substance 6-hydroxydopamine, which subsequently leads to the damage of the nigrostriatal pathway that connects the substantia nigra with the striatum. The latter is responsible, among others, for planning body movements. Damage of this area underlies PD. Tangeretin, a flavonoid that also belongs to the flavone subclass, given to mice passes the blood-brain barrier and protects the nigrostriatal pathway against the adverse effects of 6-hydroxydopamine. The significant protection of striatonigral integrity and functionality by tangeretin suggests its potential use as a neuroprotective agent (Datla et al. 2001). In addition, nobiletin, another substance belonging to the flavone group, blocks the signaling pathways involved in platelet activation by inhibiting the PLCγ2/PKC cascade and hydroxyl radical (•OH) formation and, subsequently, suppresses the activation of Akt and MAPKs. These alterations reduce Ca^{2+} concentrations and inhibit platelet aggregation. These findings suggest that

nobiletin represents a novel therapeutic agent for the prevention or treatment of thromboembolic disorders (Lu et al. 2016).

Luteolin protects against vascular inflammation in animal and human endothelial cell culture study. Dietary supplementation with luteolin (0.6% luteolin) reduces tumor necrosis factor alpha (TNF-α)-induced vascular inflammation in C57BL/6 mice (Jia et al. 2015). Luteolin intake prevented TNF-α-induced aortic structural changes in the intimal layer of artery and disruption of aortic elastin fibers (Jia et al. 2015). Furthermore, in vivo pretreatment with luteolin (as low as 0.5 μM) inhibited TNF-α-induced binding of monocytes to endothelial cells, whereas 20 μM of luteolin completely blocked monocyte adhesion. Moreover, treatment of the cells with luteolin at a concentration of 0.1 μM inhibited TNF-α-induced gene expression of chemokine monocyte chemotactic protein-1 (MCP-1) and intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), while 2 μM of luteolin almost completely prevented the stimulated expression of these proinflammatory molecules by TNF-α (Jia et al. 2015). Importantly, luteolin inhibits TNF-α-induced activation of NF-κB signaling pathway (Jia et al. 2015), suggesting that luteolin may be a novel agent for the protection against vascular inflammation. Luteolin is also proposed to be a promising agent in the treatment of epilepsy (Zhen et al. 2016). Pretreatment of pentylenetetrazol-induced epileptic rats with luteolin (50 or 100 mg/kg/day by oral administration) suppressed seizure induction and duration, reversed cognitive impairment, and reduced neuronal and oxidative stress damage. The authors observed that luteolin administration increased phosphoactivation of the protein kinase A (PKA) and cAMP response element-binding protein (CREB) as well as BDNF expression (Zhen et al. 2016).

Luteolin-6-C-β-D-boivinopyranosyl-3′-O-β-D-glucopyranoside and chrysoeriol-6-C-β-D-boivinopyranosyl-4′-O-β-D-glucopyranoside, flavones isolated from *Alternanthera philoxeroides*, an aquatic plant from South America, have significant anti-hepatitis B virus activities. These substances inhibit the secretion of HBsAg from the HepG2.2.15 cells. Thus, botanical agents may be attractive sources of a new class of anti-HBV drugs (Li et al. 2016a). Flavones exert beneficial effects on the mechanisms responsible for triggering diabetes. In a study performed in women (n = 1997), intakes of these flavonoid subclasses are inversely associated with biomarkers of inflammation, insulin concentration, and the HOMA-IR index. Participants with high intakes of flavones had lower HOMA-IR and insulin concentrations and higher adiponectin concentrations, with respective differences of −0.1 μg/ml (P-trend = 0.04), −0.5 μg/ml (P-trend = 0.02), and 0.6 μg/l (P-trend = 0.01) comparing extreme quintiles of intake (Jennings et al. 2014). Similarly, the inverse associations between flavone and flavonol intake, insulin resistance, and insulin level are also present in the Korean National Health and Nutrition Examination Survey (4186 men and women), but only in men (Yeon et al. 2015).

Flavones stimulate adipocyte differentiation and enhance glucose transport in mouse adipocyte cell line (3T3-L1 adipocytes) by induction of PPARγ-mediated expression of adiponectin and translocation of GLUT4 (Liao et al. 2012). Flavones may also have anticancer properties. A meta-analysis of twelve prospective cohort or case-control studies involving 9513 cases and 181,906 controls shows that the

risk of breast cancer is significantly lower in women with high intake of flavonols (RR = 0.88, 95% CI 0.80–0.98) and flavones (RR = 0.83, 95% CI, 0.76–0.91) compared with females at low intakes of flavonols and flavones, but not other flavonoid subclasses or total flavonoids (Hui et al. 2013). In summary, regular intake of flavones can exert beneficial effects in the prevention and treatment of various NCDs, including diabetes, cancer, PD, chronic hepatitis, and epilepsy.

14.4.5 Flavonols

Kaempferol is a substance isolated from the flavonols subclass and recently reported that this flavonoid possesses antiproliferative and apoptosis-inducing activities in several cancer cell lines, including human cervical cancer cells (SiHa and Hela), human osteosarcoma cells, cholangiocarcinoma cells, human breast carcinoma (MCF-7) cells, human bladder cancer cells (5637, T24), human stomach carcinoma cells (SGC-7901), and human lung carcinoma cells (A549) (Chen et al. 2013, Dang et al. 2015, Kim et al. 2016b, Li et al. 2015, Liao et al. 2016, Qin et al. 2016, Tu et al. 2016). Kaempferol is a potential therapeutic agent for cancer metastasis (Li et al. 2015). The anti-metastatic action of kaempferol is related to its ability to inhibit matrix metalloproteinases (MMPs) that assist tumor cells during metastasis, by inducing excessive degradation of the extracellular matrix, and thus facilitation of cancer cells invasion (Jablonska-Trypuc et al. 2016). Kaempferol blocks lung metastasis of B16F10 murine melanoma cells, as well as downregulating the expression and activity of MMP-9 in vivo. Moreover, kaempferol inhibits cancer cell invasion by inhibiting the activation of the transcription factor, activator protein-1 (AP-1), and the MAPK signaling pathway (Li et al. 2015). In the human osteosarcoma U2OS cell line, kaempferol treatment decreases DNA binding of AP-1 via attenuation of extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38-MAPKs signaling pathways and reduces enzymatic activities and protein levels of MMP-2, MMP-9, and urokinase plasminogen activator (uPA) (Chen et al. 2013). This is an important observation, as many structurally similar flavonoid compounds have no such effects on AP-1 and this suggesting that kaempferol may be a novel anticancer agent. More detailed studies are needed to support the potential effects of kaempferol as an anticancer agent for clinical use.

Quercetin has a wide range of biological actions, including anti-inflammatory, antidiabetic, antihypertensive, and antiviral activities. This compound has significant effects on the pharmacokinetics of ritonavir in rats and markedly enhances its distribution into the brain in vivo. A new strategy of treatment that might result in higher brain distribution, reduced toxicity, and improved efficacy of HIV-1 protease inhibitors, which are key components of the highly effective antiretroviral therapy, currently used to treat HIV-1 infection. Among the eight flavonoids, quercetin exerts the strongest effect on the accumulation of ritonavir in human brain-microvascular endothelial cells, with an increase of 77% (Liang et al. 2016). Quercetin protects cognitive and emotional function in an aged triple transgenic AD mouse model

where treatment (25 mg/kg body weight/every 48 h for three consecutive months beginning at 18–21 months of age) reversed β-amyloidosis, decreased tauopathy, and reduced astrogliosis and microgliosis (Sabogal-Guaqueta et al. 2015). Natural flavonols (myricetin, quercetin, kaempferol, and morin) and one flavone (apigenin) inhibit beta-site APP cleaving enzyme-1 (BACE-1) activity in both a cell-free system and in neuronal cells (Shimmyo et al. 2008). Each flavonoid reduces levels of amyloid β peptide-40 (Aβ1-40) and Aβ1-42 in neurons. Aβ is the major and core component of senile plaques and is neurotoxic. Aβ is generated from the amyloid precursor protein (APP) by BACE-1 (β-secretase) and γ-secretase. Natural flavonoids may induce neuronal Aβ reduction possibly by BACE-1 inhibition (Shimmyo et al. 2008).

Quercetin promotes cholesterol conversion to bile acids and causing a consequent cholesterol efflux. Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the production of bile acids. Dietary supplementation of quercetin (0.04% in diet) increases the mRNA and protein expression levels of CYP7A1 in the liver. Furthermore, the total bile acid contents of feces and bile in the quercetin-supplemented group are higher, whereas the dry weight of feces and bile excretion are similar between treated and control groups. These data suggest that quercetin treatment increases total excretion of bile acids (Zhang et al. 2016). Quercetin is also a potentially effective agent for the treatment of pulmonary arterial hypertension. Quercetin reverses the excessive proliferation and apoptotic resistance of pulmonary artery smooth muscle cells exposed to hypoxia, at least in part through modulation of the TrkA/AKT signaling pathway (He et al. 2015). Quercetin can also have cardioprotective properties by virtue of its anti-inflammatory, antioxidant, and anti-apoptotic effects, which protects against myocardial damage in rats (Li et al. 2016b). Compared with the control group, quercetin decreases mRNA and protein levels of TNF-alpha and IL-1beta and also MDA content and increases superoxide dismutase (SOD) and catalase (CAT) activities in the myocardial tissue of rats in both the low-dose (100 mg quercetin/kg) and high-dose (400 mg quercetin/kg) groups (Li et al. 2016b).

There are several epidemiological studies that support beneficial effect of flavonols. The meta-analysis of 13 prospective cohort studies, consisting of 344,488 subjects with 12,445 CVDs cases, shows an inverse association between flavonol intake and CVD risk. An analysis of the dose-response indicated that an average increase of 10 mg of flavonol intake per day causes a 5% lower risk of CVDs (Wang et al. 2014). However, while most of the studies report favorable associations between the intake of quercetin and decreased risk of NCDs, a few investigations do not support this observation. For example, in a small randomized, double-blind, placebo-controlled, crossover trial, quercetin-3-glucoside supplementation (160 mg/day) had no effect on flow-mediated dilation, insulin resistance, or other CVD risk factors (Dower et al. 2015). However, this human study had a small sample size ($n = 37$). Thus, further investigation is required to clarify whether quercetin can be used to prevent and treat NCDs. Collectively, flavonols seem to be useful agents in cardiovascular and anticancer prevention, can improve efficacy of antiviral treatment with ritonavir, and may protect against pulmonary arterial hypertension and AD.

14.4.6 Isoflavones

A number of human, animal, and cell culture studies reported the potential benefits of genistein in breast, prostate, and ovarian cancers. The mechanism of genistein influence on cancer is complex and requires detailed investigation. It is not always clear if and when the same molecule exerts adverse or beneficial effects. Breast cancer is classified into three large subtypes: triple negative BC (TNBC, which do not express estrogen, progesterone, or HER2 receptors), HER2, and ER expressing subtypes. A systematic review shows that genistein plays an important role in this triple scenario (Russo et al. 2016). Some studies report favorable effects of both high and low doses of genistein as they decrease proliferation of ERα-β and HER2 breast cancer cells (Jiang et al. 2013, Prietsch et al. 2014, Seo et al. 2011). On the other hand, high doses can increase markers of proliferation, such as fibroblast growth factor receptor 2 (FGFR2), transcription factor (E2F5), budding uninhibited by benzimidazoles 1 (BUB1), cyclin B2 (CCNB2), MYB proto-oncogene like 2 (MYBL2), cell division cycle protein 20 (CDC20), and CDK1 (Shike et al. 2014). The adenosine triphosphate (ATP)-binding cassette G2 efflux transporter, which is also called the breast cancer resistance protein (BCRP), mediates the disposition and excretion of numerous endogenous chemicals and xenobiotics. Genistein and daidzein and their metabolites interact with BCRP as substrates, inhibitors, and/or modulators of gene expression. When acting as BCRP inhibitors, these isoflavones promote reversion of multidrug resistance and sensitize cancer cells to chemotherapy. However, negative outcomes can also occur, such as enhancement of the toxicity or a decrease in the efficacy of a specific drug (Bircsak and Aleksunes 2015).

In general, the prevalence of the breast cancer is lower in Asian than in North American and European countries (Jemal et al. 2011). This may be related to a higher intake of soy, that is, an abundant source of isoflavones. In prospective observational studies in Asian populations, soy intake was associated with a reduced risk of breast cancer. A dose-response relationship shows a significant decrease in the risk of breast cancer with the increases in soy food intake, translating to a 16% risk reduction per every 10 mg of daily isoflavone consumed (Wu et al. 2008). A meta-analysis of 22 observational studies shows that isoflavone intake reduces breast cancer risk (a combined relative risks and odds ratios of 0.68, 95% confidence interval, 0.52–0.89) in Asian populations but not in Western populations (a combined relative risks and odds ratios of 0.98, 95% confidence interval, 0.87, 1.11) for the high-dose category. The protective effect of isoflavones in Asian participants was evident in concerned both pre- and postmenopausal women (Xie et al. 2013). On the other hand, a population-based prospective cohort study in Japan (15,607 women) shows a beneficial effect only in postmenopausal females. The relative risks of postmenopausal breast cancer are lower in women with higher intakes of soy (trend $p = 0.023$) and isoflavones (trend $p = 0.046$), although the relative risks of premenopausal breast cancer are not associated with intakes neither of soy nor isoflavones. The authors observed a reduced risk of postmenopausal breast cancer even in women with moderate intake of soy (hazard ratio, 0.65 in the second quartile) and isoflavones

(hazard ratio, 0.57 in the second quartile) (Wada et al. 2013). These results suggest that soy and isoflavone intakes have a protective effect against postmenopausal breast cancer in Asian populations, but this effect is different in various populations.

14.5 Conclusions

Epidemiological, animal, and in vitro studies strongly support a positive effect of flavonoids on health maintenance. This applies to flavonoids consumed both in regular diets and with supplements. These compounds have a wide range of biological actions, including anti-inflammatory, antioxidant, antiproliferative, anti-angiogenic, antidiabetic, antihypertensive, antiplatelet, and antiviral activities. Furthermore, their consumption improves postprandial hyperglycemia, protects LDL particles from oxidative modification, reduces neuroinflammation, and improves lipid profile in hyperlipidemic patients. However, there is a great need for a better understanding of the underlying molecular mechanisms and also their metabolism in humans.

Flavonoids play a beneficial role in disease prevention and treatment; however, knowledge of the potential side effects of supplements that contain supraphysiological quantities of specific flavonoid compounds is scarce. Further investigations, especially clinical randomized trials, focusing on both the effectiveness and safety of flavonoid use, are greatly needed. Our current knowledge support the recommendation to consume flavonoids in foods, such as fruits and vegetables, legumes, nuts, spices, and herbs every day, with suggested daily intake of 600 g of fruits and vegetables. There is also much support for recommending the Mediterranean diet (MD) and/or the Dietary Approaches to Stop Hypertension (DASH) diet as they are both rich in flavonoids which have been shown to maintain good health and prevent NCDs.

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Chapter 15
Dietary Polyphenols in the Prevention and Treatment of Diabetes Mellitus

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Abstract Type 2 diabetes mellitus (T2DM) is an auto-inflammatory disease that is associated with oxidative stress and insulin resistance. The increased production of reactive oxygen species (ROS) or a reduced capacity of the ROS scavenging antioxidants can lead to abnormal changes in intracellular signalling and result in chronic inflammation and insulin resistance. Mediators of oxidative stress and inflammation activate various transcriptional and metabolic pathways that lead to T2DM pathogenesis and its associated complications. Prevention of ROS-induced oxidative stress and inflammation can be an important therapeutic strategy to prevent the onset of T2DM and diabetic complications. A healthy diet is a major lifestyle factor that can greatly influence the incidence and development of T2DM. Polyphenols, the most abundant antioxidants in the diet, have attained considerable interest due to their potential pharmacotherapeutic properties and are believed to promote health and reduce the risk of non-communicable diseases including T2DM. The main focus of this chapter is to provide an overview regarding the dietary polyphenols and their antioxidant properties and possible roles in the prevention and treatment of T2DM and its associated complications.

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15.1 Introduction

Type 2 diabetes mellitus (T2DM) is a chronic inflammatory/autoimmune disease (Donath and Shoelson 2011) that is increasing on a global scale. It is generally agreed that T2DM is accompanied by the persistent occurrence of hyperglycaemia and hyperlipidaemia; however, the risk factors that lead to these disorders are unknown to many of those affected. Several studies have reported that mediators of oxidative stress and pro-inflammation are involved in the activation of various transcriptional and metabolic pathways that ultimately lead to hyperglycaemia, hyperlipidaemia and insulin resistance (Akash et al. 2013a, b, c).

Expensive pharmacotherapy has failed to stem the worldwide rise in non-communicable diseases (NCDs) importantly diabetes; therefore there is an urgent need for inexpensive preventive strategies (Prasad et al. 2015). Preventable unhealthy lifestyle behaviours, such as poor nutrition (food with high content in free sugars and fats) and physical inactivity, are risk factors of chronic non-communicable diseases including T2DM (Yang et al. 2011). A healthy diet is a major lifestyle factor that can prevent the incidence and development of T2DM (Ríos-Hoyo et al. 2014; Kahleova and Pelikanova 2015; Sluijs et al. 2015; Kimokoti and Millen 2016). Polyphenols, important constituents of fruits and vegetables, plant-derived beverage, and food products of plant origin, are well recognized as natural antioxidants that might explain their presumed therapeutic potential in NCDs prevention (Arts and Hollman 2005; Ullah and Khan 2008; Pandey and Rizvi 2009; Ríos-Hoyo et al. 2014; Thenmozhi et al. 2016).

Epidemiological studies indicate that a diet rich in polyphenolic compounds can prevent T2DM (Montonen et al. 2004; Mirmiran et al. 2009, 2012; Pandey and Rizvi 2009; Bahadoran et al. 2012, 2013). The widespread presence of polyphenols in the human diet and apparent low toxicity suggest that polyphenols have the potential to protect health against oxidative stress and associated NCDs (Pandey and Rizvi 2009), including T2DM (Xiao and Hogger 2015). This chapter reviews dietary polyphenols and their possible roles in the prevention and treatment of T2DM and its associated complications.

15.2 Dietary Polyphenols

Polyphenols are an important class of phytochemicals and secondary plant metabolites that are abundantly present in variety of fruits, vegetables and nuts (Lapornik et al. 2005; Tomás-Barberán et al. 2000). Polyphenols have an aromatic ring containing one or more hydroxyl groups. Therefore, the structure of polyphenols may vary from a simple phenolic molecule to complex molecules with high molecular

mass (Balasundram et al. 2006). Polyphenols are classified into several major categories depending upon the number of phenolic rings they contain and the structures that bind to these phenolic rings. We discuss the most important classes of dietary polyphenols below.

15.2.1 Flavonoids

Flavonoids exist as α or β glycosides in plants (Fang et al. 2013; Pugliese et al. 2013; Taheri et al. 2013). Flavonoids are low molecular weight phytochemicals containing 15 carbon atoms arranged in a C6-C3-C6 configuration with two aromatic rings in their structure joined by a 3-carbon bridge; however, variations in the substitution pattern of ring C results in a subclassification of dietary flavonoids. In isoflavones, hydroxyl groups are present at C4 and C7, and their structures are similar to that of oestradiol molecule. They are mainly found in plants and plant-derived foods. In flavanones, a saturated three-carbon chain and oxygen atom are present at C4, whereas the glycosylation occurs at C7. Flavanones are water-soluble pigments that are present in all parts of the plant including flowers, leaves, fruits, stems and roots.

15.2.2 Tannins

Tannins are an important group of dietary polyphenols that exhibit diverse therapeutic effects in various biological systems (Kumari and Jain 2015). Tannins are subclassified into four major groups, gallotannins, ellagitannins, complex tannins and condensed tannins, on the basis of their structural characteristics (Khanbabaee and van Ree 2001). Gallotannins are all those tannins in which galloyl units or their meta-depsidic derivatives are bound to diverse polyol, catechin or triterpenoid units. Ellagitannins are those tannins in which at least two galloyl units are C-C coupled to each other and do not contain a glycosidically linked catechin unit. Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit. Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of C4 of one catechin with C8 or C6 of the next monomeric catechin.

15.2.3 Phenolic Acids

Phenolic acids are present in free and bound forms (Robbins 2003). The later acids are linked to various plant components through acetal, ester and ether bonds (Zadernowski et al. 2009). Phenolic acids are further subclassified into two subgroups including hydroxybenzoic acid and hydroxycinnamic acid. Hydroxybenzoic acids are a subclass of phenolic acids and contain a C6-C1 structure. The most common examples of hydroxybenzoic acids are p-hydroxybenzoic acid, gallic acid,

vanillic acid, protocatechuic acid and syringic acid. Hydroxycinnamic acids are aromatic compounds with a three-carbon side chain (C6-C3). Sinapic acid, p-coumaric acid, ferulic acid and caffeic acid are common representatives of hydroxycinnamic acids. Stilbenes exist in the form of monomers and/or oligomers, which are characterized by the presence of 1,2-diphenylethylene nucleus with hydroxyl groups that are substituted on aromatic rings. One of the best-known compounds is trans-resveratrol that contains trihydroxystilbene skeleton in its structure (Han et al. 2007). The curcumin diferuloylmethanes are small group of phenolic compounds that contain two aromatic rings substituted with hydroxyl groups. These are linked with aliphatic chain containing carbonyl group (Schaffer et al. 2007; Tuck et al. 2001).

15.3 Bioavailability of Dietary Polyphenols

Health benefits of polyphenols depend on food sources, amount in diet, intestinal absorption, intestinal and hepatic metabolism, transport, bioavailability for organs and tissues of various biological systems, cellular uptake and intracellular metabolism. Moreover, most of polyphenols occur as glycosylated derivatives in plants and foods, and the bioactivity of dietary polyphenols depends on diverse intestinal transformations by digestive and/or gut microflora enzymes (Marín et al. 2015). In addition, bioavailability of dietary polyphenols differs for various types of phenolic compounds (Manach et al. 2004, 2005). After absorption, most of the dietary polyphenols, notably flavonoids, are converted into their corresponding glucuronide conjugates in the intestinal epithelium (Marvalin and Azerad 2011; Williamson 2002) after which they enter the systemic circulation. Once absorbed, dietary polyphenols reach the systemic circulation after conjugation with glucuronide, methyl and sulphate groups in gut mucosa, whereas non-conjugated dietary polyphenols do not reach the systemic circulation (Han et al. 2007). It is important to note that before absorption of polyphenols, only a small amount of them are being absorbed directly from gastrointestinal tract and reach the target organs. Indeed, dietary polyphenols are metabolized and used by beneficial gut bacteria for their growth, proliferation and survival (Selma et al. 2009; Marín et al. 2015). Unlike the native polyphenols, their metabolites may have different biological activities in organs and tissues of biological systems.

15.4 Antidiabetic Effects of Dietary Polyphenols

Diabetes mellitus is a global public health issue, and treatment strategies using current conventional antidiabetics can have side effects. In view of these limitations, scientists aim to discover the plant-based phytochemical constituents that may have a potential to combat diabetes mellitus. Dietary polyphenols have diverse pharmacotherapeutic properties against diabetes mellitus in large part due to their

antioxidant, anti-inflammatory and chemopreventive activities. Given that oxidative stress contributes to development of diabetes complications (Baynes 1991), considerable efforts have been focused on antioxidant therapies, including the use of polyphenols, in reducing the risk of diabetes (Crespy and Williamson 2004; Scalbert et al. 2005).

The increased production of cellular reactive oxygen species (ROS), mainly superoxide anion ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$), or a reduced capacity of ROS scavenging antioxidants can lead to abnormal changes in intracellular signalling and result in chronic inflammation and insulin resistance. Oxidative stress exhibits its damaging effects on various organs through the generation of ROS causing an imbalance between antioxidant and oxidant statuses in diabetic conditions (Akash et al. 2012b, 2013b). The demonstration of antioxidant and antidiabetic potentials of polyphenols, including flavonoids, tannins and phenolic acids, in experimental animal models and/or epidemiological studies is encouraging.

Animal studies performed in vivo and in vitro confirm that dietary flavonoids have beneficial effects on glucose regulation, carbohydrate metabolism, glucose uptake and insulin secretion through the activation of various intracellular signalling pathways (Cai and Lin 2009; Fu et al. 2010; Hanhineva et al. 2010; Takikawa et al. 2010; Zhang and Liu 2011). Meta-analysis and epidemiological studies report an inverse relationship between the consumption of dietary flavonoids and development of diabetes mellitus and its associated complications (Arts and Hollman 2005; Graf et al. 2005). The antidiabetic effects of these compounds have been investigated using various experimental animal models, in vitro studies and epidemiological studies (Cai and Lin 2009; Green et al. 2011; Jung et al. 2004; Kobori et al. 2009; Mahmoud et al. 2012; Ortsater et al. 2012; Tsuda et al. 2003; Zhang et al. 2011). These studies indicate that flavonoids have antidiabetic effects and can prevent T2DM.

Tannins have received considerable interest in health promotion due to their nutraceutical properties (Kunyanga et al. 2011). In experimentally induced diabetes mellitus, tannins improve blood glucose, lipid profiles and restored serum levels of insulin and low-density lipoproteins (Babby et al. 2014; Kunyanga et al. 2011; Liu et al. 2005; Velayutham et al. 2012). Moreover, tannins have significant antioxidant activities (Kunyanga et al. 2011; Velayutham et al. 2012) and stimulate various molecular and metabolic pathways to restore normoglycaemia (Liu et al. 2005).

Diet-based phenolic acids are among the most important phenolic compounds with antidiabetic properties (Ali Asgar 2013; Vinayagam et al. 2016). Data from studies in diabetic animal models indicate a role for phenolic acids in the prevention and treatment of diabetes mellitus (Jung et al. 2007). Postprandial hyperglycaemia, an important causative factor responsible for the development of T2DM, which is regulated by starch digestive enzymes, is an important strategy to control the postprandial hyperglycaemia (Lo Piparo et al. 2008; Obiro et al. 2008). Phenolic acids can inhibit digestive enzymes for the metabolism of starch (Shobana et al. 2009).

15.5 Protective Effects of Dietary Polyphenols Against Oxidative Stress-Induced Diabetes Mellitus

Diabetes-associated complications have the potential to be life-threatening, and the prevention of diabetes-associated complications remains a challenge for medical practitioners. Several studies suggest that dietary polyphenols have the ability to prevent and cure several diabetes-induced complications (Cui et al. 2008; Ghosh and Konishi 2007; Li et al. 2008). Dietary polyphenols have the potential in management of diabetes (Bahadoran et al. 2013). Most phenolic compounds and their active metabolites have been reported to have potent antioxidant properties. These phytochemical constituents attenuate the generation of ROS and hydroperoxide formation and quench electronically excited compounds through the modulation of enzymes such as cyclooxygenase (COX), mitochondrial succinoxidase, microsomal monooxygenase, lipoxygenase (LOX), nicotinamide adenine dinucleotide phosphate (NADH) oxidase and xanthine oxidase (XO) (Dembinska-Kiec et al. 2008). Polyphenols, like resveratrol and anthocyanins, protect β-cells of the islets of Langerhans in the pancreas against oxidative stress and cell apoptosis and thus improved glucose tolerance (Zhang et al. 2010; Szkudelski and Szkudelska 2011), suggesting that dietary polyphenols may be used as alternate treatment strategies for the management of oxidative stress-induced diabetic complications.

15.5.1 Protective Effects Against Diabetes-Associated Cardiovascular Complications

Insulin resistance is a major hallmark of T2DM and is mainly responsible for progressive cardiovascular risk factors that lead to the development of atherosclerotic coronary artery disease and other cardiovascular complications (Kalofoutis et al. 2007). Likewise, dyslipidaemia, which also occurs in T2DM, is responsible for vascular endothelial and smooth muscle cell damage (Thomas and Foody 2007). There is growing evidence to suggest that dietary polyphenols and their supplements have protective effects on diabetes-associated cardiovascular complications by modulating the regulation of lipid metabolism.

Polyphenols prevent cholesterol accumulation in human macrophages (Sevov et al. 2006). Tea catechins decrease the levels of cholesterol and triglyceride in the rat aorta (Miura et al. 2001). Fruit polyphenols, like hesperidin and naringenin, reduce the plasma cholesterol levels, LDL cholesterol and triglycerides in hyperlipemic rat model (Monforte et al. 1995; Lee et al. 1998). The endothelial $NO^{\bullet}$ production promotes endothelium-dependent vasorelaxation. $NO^{\bullet}$ is generated by endothelial nitric oxide synthase (eNOS) through the conversion of L-arginine to L-citrulline. Black currants (*Ribes nigrum* L.), with high content of polyphenolic compounds, enhance synthesis of $NO^{\bullet}$ in the rat aorta and induce the endothelium-dependent vasorelaxation via the H1 receptors on the endothelium (Nakamura et al.

2002). Resveratrol, a polyphenol found largely in red grape skin, increases eNOS activity and leads to vasodilatation through augmenting synthesis of $NO^{\bullet}$ in the rat heart (Das et al. 2005).

15.5.2 Protective Effects Against Diabetes-Associated Adipose Tissue Dysfunction

Dysfunction of adipose tissues is associated with the impairment of β-cell function and insulin secretion, development of insulin resistance and induction of inflammation that ultimately lead to the development of T2DM and its complications (Guilherme et al. 2008). Dietary polyphenols increase the β-oxidation in adipose tissues and downregulate the genes and enzymes that are involved in lipogenesis (Nakazato et al. 2006; Osada et al. 2006) and prevent the dysfunctioning of adipocytes (Tsuda et al. 2003, 2006). The transcriptional factor sterol regulatory element-binding protein 1c (SREBP-1c) enhances lipogenesis and adipogenesis (Wakil and Abu-Elheiga 2009). The tea catechin, epigallocatechin gallate (EGCG), represses activity of glycerol-3-phosphate dehydrogenase, an enzyme involved in lipid synthesis; decreases activity of SREBP-1c, an enhancer of lipogenesis and adipogenesis; and reduces glucose and fatty acid transport and intracellular lipid accumulation (Kim et al. 2010).

15.5.3 Protective Effects Against Diabetes-Associated Neurodegenerative Diseases

Glucose homeostasis is vital to the functioning of the nervous system, since glucose is the exclusive energy source required for neuronal activity. Diabetes mellitus has long been shown to be associated with dementia (Perlmuter et al. 1984; Richardson 1990), and both T2DM and Alzheimer's disease (AD) share common abnormalities including impaired glucose metabolism, increased oxidative stress, insulin resistance and amyloidogenesis (Zhao and Townsend 2009; Vignini et al. 2013). Hyperinsulinaemia and T2DM are among the risk factors of AD (Neumann et al. 2008). Cerebral cortex, hypothalamus and cerebellum of 8-week diabetic rats presented abnormal morphologic structure associated with basal depressive behaviour as evidenced by decreased struggle time and ambulatory activity (Hernandez-Fonseca et al. 2009).

The incidence of dementia increases in later life and T2DM may be a risk factor for cognitive dysfunction, particularly those related to AD (Umegaki 2012). Amyloid beta (Aβ or Abeta) plays a determinable role in the onset of AD. Although insulin has important functions in the brain, experimental studies indicate that insulin accelerates Alzheimer-related pathology, at least in part, through its effects on the Abeta metabolism (Umegaki 2012). Furthermore, deposits of Abeta produce a loss of neu-

ronal surface insulin receptors and directly interfere with insulin signalling pathway (Vignini et al. 2013). In addition, Abeta can generate ROS in the presence of the transition metals copper and iron in vitro (Smith et al. 2007). Oxidative stress induced by H_2O_2 (100–250 mM) increases the levels of Abeta in human neuroblastoma SH-SY5Y cells (Misonou et al. 2000). More recently, high glucose and Abeta reduce cell viability and enhance mitochondrial ROS production in rodent brain endothelial cells supporting the role of hyperglycaemia in vascular injury associated with AD (Carvalho et al. 2014). Experimental studies suggest that diet-based antioxidant therapy reduces ROS-induced diabetic neurodegenerative disorders (Bajaj and Khan 2012; Birben et al. 2012), although convincing evidence for clinical efficacy is still lacking.

15.5.4 Protective Effects Against Diabetes Associated Nephropathy

Diabetic nephropathy, a microvascular complication of diabetes, is a late diabetic complication leading ultimately to renal failure. Growing evidence suggests that ROS may play an important role in the initiation and progression of diabetic nephropathy and renal dysfunction (Coughlan et al. 2008; Kashihara et al. 2010), which is responsible for significant causes of morbidity and mortality. Removal of ROS by antioxidants may be an effective therapeutic strategy for prevention of diabetic nephropathy. ROS-mediated podocyte apoptosis represents an early glomerular cell defect associated with glomerulopathy in murine type 1 and type 2 diabetic models (Susztak et al. 2006). Dietary polyphenols having potent antioxidant capacity are the opted treatment strategy to prevent the production of ROS and diabetes-induced nephropathy (Cui et al. 2008; Li et al. 2008). Treatment of diabetic nephropathy model rats with (−)-epigallocatechin 3-O-gallate alleviates renal damage caused by abnormal glucose metabolism-associated oxidative stress (Yamabe et al. 2006).

15.6 Mechanism of Action of Dietary Polyphenols

Metabolic syndrome, encompassing hyperglycaemia and impaired glucose tolerance, is the major predisposing factor of T2DM (Eckel et al. 2005). One of the defects in metabolic syndrome and its associated diseases is an imbalance between the production and removal of ROS (Roberts and Sindhu 2009). Glucose metabolism disturbance leads to an increase of fat mass and storage in the body resulting in inflammation, oxidative stress and malfunction of several tissues and organs including muscle, adipose tissue, liver and pancreas (Lann et al. 2008). Dietary polyphenols can influence glucose metabolism by stimulating peripheral glucose uptake in insulin-sensitive and non-insulin-sensitive tissues (Hanhineva et al. 2010) and

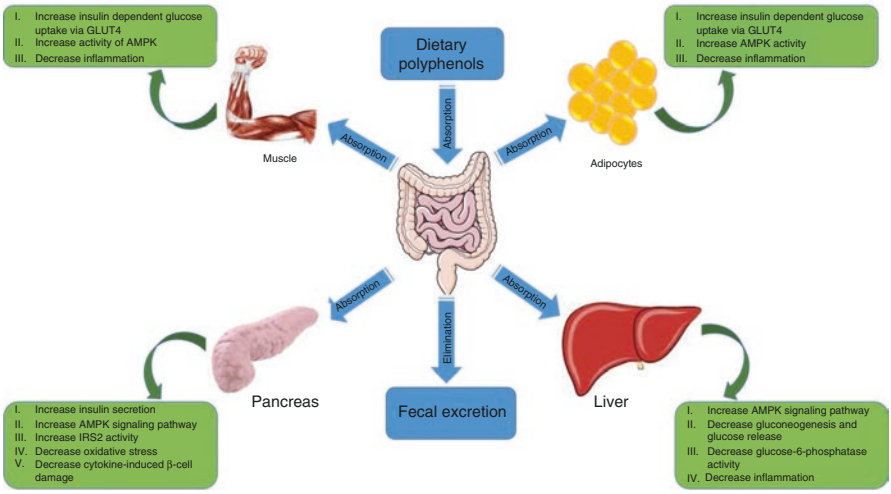

Fig. 15.1 Schematic representation for the mechanism of action of dietary polyphenols to improve glucose homeostasis. *GLUT4* glucose transporter 4, *AMPK* adenosine monophosphate-activated protein kinase, *IRS2* insulin receptor substrate 2

improve glucose homeostasis (Fig. 15.1). After absorption from intestinal tract, dietary bioavailable polyphenols are entered into the bloodstream and exhibit their effects on organs and tissues of biological systems. In the following subsections, we have classified the mechanism of action of dietary polyphenols by which they improve glucose homeostasis in the muscle, adipose tissue, liver and pancreas.

15.6.1 Improvement of Glucose Homeostasis in Muscles

Diabetes mellitus is characterized by the low-grade inflammation in β-cells of pancreatic islets, adipocytes and peripheral tissues (Akash et al. 2012a, b, 2013a, b, c, d). Inflammation in peripheral tissues is responsible to decrease the insulin sensitivity due to which glucose uptake in peripheral tissues is decreased. It has been shown that high concentrations of H_2O_2 activate insulin signalling and induce the typical metabolic actions of insulin (Czech et al. 1974) and hence increase glucose uptake in muscle cells (Higaki et al. 2008). Resveratrol improves glucose uptake in skeletal muscle cell line C2C12 myoblasts via activation of glucose transporter 4 (GLUT4), and adenosine monophosphate activated the AMP-activated protein kinase (AMPK) signalling pathway protein kinase (Park et al. 2007). Resveratrol also increases the expression of GLUT4 in the rat muscle tissue by activation of the phosphatidylinositol-3 kinase (PI3K) pathway, which mediates GLUT4 translocation and glucose transport (Chi et al. 2007). Dietary polyphenols increase the sensitivity of insulin in peripheral tissues by decreasing the inflammation in peripheral tissues (Fig. 15.1).

15.6.2 Improvement of Glucose Homeostasis in Adipocytes

Adipocytes are the main source of adipocytokines. Leptin, which behaves as pro-inflammatory cytokine, and adiponectin that acts as anti-inflammatory factor, are important adipocytokines for glucose homeostasis in adipocytes. The imbalance between adiponectin and leptin may induce inflammation in adipocytes (Otero et al. 2005), decreasing the activity of GLUT4 and AMPK. This decrease in GLUT4 activity can disrupt the utilization of insulin-dependent glucose. Of note, activation of insulin signalling by H_2O_2 increases glucose uptake and stimulates GLUT4 translocation and lipid biosynthesis in rat adipocytes (May and de Haen 1979). Dietary polyphenols increase the insulin-dependent glucose utilization via activation of GLUT4 and AMPK. Dietary polyphenols may also decrease the inflammation in adipocytes by balancing the levels of leptin and adiponectin (Fig. 15.1).

15.6.3 Improvement of Glucose Homeostasis in Liver

The liver plays a central role in the maintenance of glucose homeostasis. Non-alcoholic fatty liver disease (NAFLD) is common in patients with T2DM (Portillo-Sanchez et al. 2015). Oxidative stress plays an important role in the pathogenesis of NAFLD. Oxidative stress, an important mediator of NAFLD, promotes hepatic necroinflammation and fibrosis, and efforts to improve the hepatic antioxidant system could be achieved by optimizing the patient's diet or by supplementation with essential metals and/or antioxidants (Gawrieh et al. 2004). Disruption in glucose-regulating enzymes such as glucose-6-phosphatase and/or signalling pathways such as AMPK deregulates the production of glucose in the liver and ultimately leads to hyperglycaemia. Dietary polyphenols decrease the production of glucose via activation of AMPK signalling and suppression in the activity of glucose-6-phosphatase (Waltner-Law et al. 2002; Collins et al. 2007). Moreover, dietary polyphenols decrease the phenomenon of gluconeogenesis due to which the production of glucose is decreased in the liver (Fig. 15.1).

15.6.4 Improvement of Glucose Homeostasis in Pancreas

Insulin is produced from the β-cells of pancreatic islets, and the level of glucose within the blood regulates production of insulin. Abnormally augmented levels of glucose may activate the production of several types of pro-inflammatory mediators and various transcriptional pathways, which are known to be responsible for the initiation of inflammation in pancreatic islets (Akash et al. 2012b, 2013a, b). Importantly, dietary polyphenols increase insulin secretion and prevent the development of diabetic cataracts in streptozotocin-induced diabetic rats. The tea polyphenol EGCG and the buckwheat flavonoid rutin stimulate insulin receptor substrate 2

(IRS2) and AMPK signalling in rat pancreatic β-cells (Cai and Lin 2009). Therefore, dietary polyphenols increase the production of insulin by increasing the signalling of AMPK and IRS2 activity (Fig. 15.1).

15.7 Dietary Polyphenols in Clinical Studies

Systemic reviews and meta-analysis reported that daily consumption of fruit and vegetable, which are rich sources of polyphenols, is inversely associated with high risk of T2DM (Carter et al. 2010; Cooper et al. 2012). Randomized, placebo-controlled trials conducted on type 2 diabetic patients showed that olive oil improves glucose metabolism, insulin sensitivity and pancreatic β-cell secretory capacity (de Bock et al. 2013; Lasa et al. 2014; Wainstein et al. 2012). Moreover, olive oil has anti-inflammatory effects as evidenced by reducing serum levels of various pro-inflammatory mediators, notably C-reactive protein, interleukin-6 and various chemokines (Estruch 2010).

Intake of whole grains is associated with decreased risk of diabetes mellitus (McKeown et al. 2002; Ye et al. 2012). Berries delay glucose absorption from intestinal tract and decrease the gastrointestinal tract motility, which may explain the reduced glycaemic response achieved by berry intake (Wilson et al. 2010; Torronen et al. 2010, 2013). It has been shown that cinnamon, a rich source of procyanidin, cinnamtannin trans-cinnamic acid, catechin and flavones (Qin et al. 2010; Sikand et al. 2015; Xiao and Hogger 2015), can improve glycaemic control, glycosylated haemoglobin and insulin sensitivity (Akilen et al. 2010; Crawford 2009; Lu et al. 2012; Qin et al. 2010; Roussel et al. 2009; Wang et al. 2007).

Cocoa and chocolate improve the insulin sensitivity and decreased fasting levels of insulin (Davison et al. 2008; Hooper et al. 2012). However, flavanol-rich cocoa drink (Muniyappa et al. 2008) or dark chocolate (Rostami et al. 2015) did not improve insulin sensitivity, fasting blood glucose and haemoglobin A1c (HbA1c) levels in diabetic patients, suggesting that chocolate cannot be recommended to control glycaemia. Although coffee can improve glucose tolerance, insulin sensitivity, glucose metabolism and insulin secretion, it is not yet proven that coffee consumption by diabetic patients can prevent T2DM (Akash et al. 2014a).

Ginger supplementation (2 g/day) for 8 weeks reduces blood insulin level and improves insulin sensitivity and lipid profile (total cholesterol, triglyceride, low-density lipoprotein and high-density lipoprotein) in type 2 diabetic patients (Mahluji et al. 2013). Supplementation with ginger (3 g/day) for 8 weeks reduces fasting blood sugar and lipid profile and HbA1c and improves insulin resistance (Mozaffari-Khosravi et al. 2014). Various studies presented in a review article (Akash et al. 2014b) show the advantage of onion consumption in the reduction of risk factors, mainly levels of blood glucose, serum lipids, oxidative stress and lipid peroxidation, associated with diabetes mellitus. We chose to present the different effects of onion in Fig. 15.2 as an example of the anti-hyperglycaemic action of dietary polyphenols.

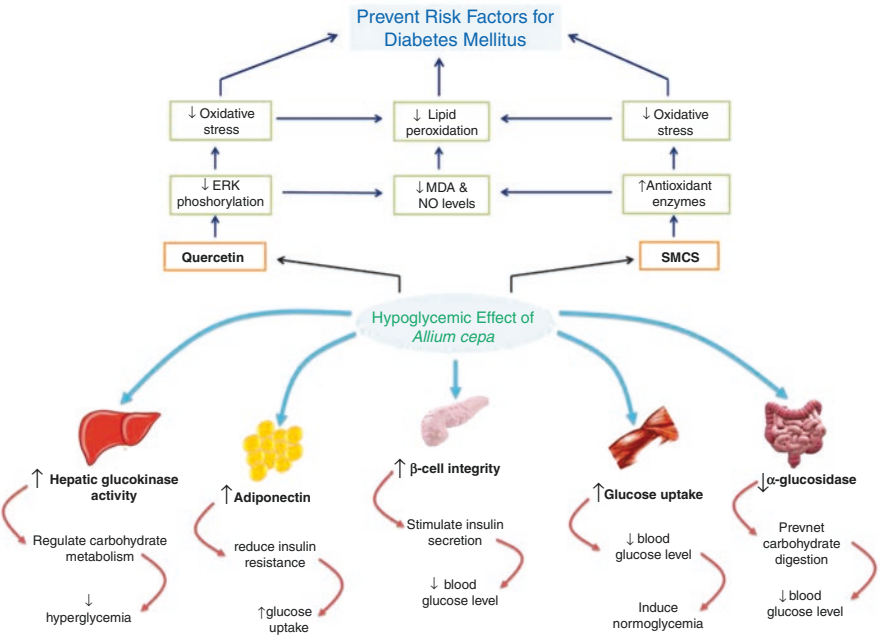

Fig. 15.2 Schematic representation of anti-hyperglycaemic effects of onion (Akash et al. 2014b). *ERK* extracellular signal-regulated kinase, *MDA* malondialdehyde, *NO•* nitric oxide, *SMCS* S-methylcysteine sulfoxide

15.8 Conclusions

Glucose is vital to the functioning of biological systems, and the factors involved in glucose homeostasis at the cellular and molecular levels are multiple and interdependent. T2DM depends on many environmental factors, importantly malnutrition and sedentary lifestyle, which share the ability to disturb glucose homeostasis. The underlying causative pathogenesis of T2DM includes inflammation and ROS-induced cellular oxidative damage. Mediators of oxidative stress and inflammation by environmental factors are involved in the activation of various transcriptional and metabolic pathways that ultimately lead to T2DM and its associated complications. Preserving the β-cells of pancreatic islets, as well as adipocytes, hepatocytes and myocytes from the damaging effects of ROS and inflammation, could be considered as an optimized treatment strategy to prevent the onset of T2DM.

There is evidence from experimental, epidemiological and clinical studies that dietary polyphenols can improve glucose homeostasis via activation of potential mechanisms of actions in various organs and tissues of biological systems, such as adipose tissue, muscles, liver and pancreas. However, still there is a need for more clinical evidence in order to support dietary recommendations aimed at the prevention of T2DM and its associated complications by dietary polyphenols. Until we get

relevant and compelling evidence for the use of polyphenols as alternate treatment strategies for the management of oxidative stress-induced diabetic complications, a healthy diet is a major lifestyle factor that can prevent the incidence and development of T2DM.

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Chapter 16
Targeting Complications of Diabetes with Antioxidants

Saeid Golbidi and Ismail Laher

Abstract Diabetes mellitus is a debilitating metabolic disorder in which oxidative stress plays a key role in the pathogenesis of the homeostatic regulation of glucose. Oxidative stress results from an imbalance between the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and defending antioxidant systems and has a major contribution in the pathogenesis of many diseases including diabetes. This has led to the promising idea that using antioxidants may be a useful therapeutic strategy. In this chapter we focus on the therapeutic role of some commonly used antioxidants including vitamin E, vitamin C, alpha-lipoic acid, L-carnitine, coenzyme Q10, and ruboxistaurin in the treatment of diabetes or its complications. We focus on the results from human clinical studies and summarize their key outcomes. Despite the promise of therapeutic benefits based on preclinical data, the results of large-scale clinical trial are inconclusive and suggest that the routine use of antioxidants may have limited or no therapeutic benefits.

Keywords Diabetes • Antioxidant • Vitamin C • Vitamin E • Coenzyme Q10 • Carnitine • Alpha-lipoic acid • Ruboxistaurin

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16.1 Introduction

Oxidative stress occurs under several conditions in the body, including during the pathogenesis of diseases such as atherosclerosis, hypertension, cancer, neurodegenerative diseases, inflammatory conditions, and diabetes and also during the normal aging process. An imbalance between the production of free radicals and other reactive metabolites, so called oxidants, and their elimination by protective mechanisms such as endogenous antioxidant enzymes results in the accumulation of highly reactive free radicals and oxidative damage. Free radicals are reactive chemical species that are oxidants; they possess a single unpaired electron in their outer orbit. They pair this unpaired electron with an electron taken from other compounds, causing their oxidation and themselves becoming reduced. A large proportion of free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are generated during normal cellular metabolism. ROS and RNS have dual deleterious and beneficial roles, since they can be either harmful or beneficial to living systems. Free radicals can also arise from deleterious environmental sources such as radiation, toxic chemicals, or cigarette smoking. The beneficial effects of ROS occur at low/moderate concentrations and involve their physiological roles in a number of cellular signaling systems, including cellular responses to anoxia, defense against infectious agents, and/or induction of a mitogenic response (Golbidi et al. 2011).

An antioxidant stabilizes or deactivates free radicals before they interact with biological systems. Humans have evolved highly complex antioxidant systems (enzymatic and nonenzymatic) that work synergistically and in combination with each other, to protect cells and organ systems against free radical-induced damage. Antioxidants can be endogenously produced substances or be obtained from exogenous sources, e.g., as a part of a diet or as dietary supplements. An ideal antioxidant should be readily absorbed and be able to quench free radicals, as well as chelate redox metals at physiologically relevant levels. Such substances should work in aqueous solutions and/or membrane domains and affect gene expression in a positive way. Endogenous antioxidants play a crucial role in maintaining optimal cellular functions and thus systemic health and well-being. However, under conditions of oxidative stress, endogenous antioxidants may not be sufficient and dietary antioxidants may be required to maintain optimal cellular functions. The most efficient enzymatic antioxidants involve glutathione peroxidase, catalase, and superoxide dismutase. Nonenzymatic antioxidants include vitamins E and C, thiol antioxidants (glutathione, thioredoxin, and lipoic acid), melatonin, carotenoids, natural flavonoids, and other compounds (Golbidi and Laher 2010).

16.2 Antioxidant Therapy in Diabetes

Diabetes mellitus is a debilitating metabolic disorder in which increased oxidative stress plays a key role in the pathogenesis of the homeostatic regulation of glucose. Consumption of antioxidant capacity secondary to an increased production of free radicals has been reported in many studies (Brownlee 2005; Bashan et al. 2009;

Rolo and Palmeira 2009). An arbitrary classification divides the origin of diabetes-induced free radicals to mitochondrial and non-mitochondrial (Brownlee 2005). Mitochondria-originated ROS can activate at least four distinct chain reactions, namely, polyol, hexosamine, protein kinase C, and advanced glycation end-product pathways (Brownlee 2005). Final products of these pathways induce pathologic changes in gene expression including decreased production of endothelial nitric oxide ($NO^{\bullet}$) synthase (eNOS) or increased expression of plasminogen activator inhibitor-1, modification of extracellular proteins, and triggering of the inflammatory cascade (Yung et al. 2006). There are some reports suggesting a decreased mitochondrial ROS production during hyperglycemia. For instance, Martens et al. (2005) reported that glucose up to 20 mm does not stimulate hydrogen peroxide (H_2O_2) or superoxide ($^{\bullet}O_2^-$) production in metabolically active beta cells, while Herlein et al. (2009) showed that there is no excess superoxide production by complexes I and III from mitochondria of streptozotocin-treated diabetic rats. It may be prudent to revisit mitochondrial hyperglycemia-induced ROS production to clarify this relationship.

Non-mitochondrial sources of ROS include nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase (XO), uncoupled eNOS, lipoxygenase, cyclooxygenase, cytochrome P450 enzymes, and other hemoproteins (Yung et al. 2006). The original role of NADPH oxidase was suggested to be in cellular defense mechanisms (Henderson and Chappel 1996). Data obtained later clarified its function in vascular smooth muscles (Lassègue and Clempus 2003), endothelial cells (Quagliaro et al. 2003), mesangial cells (Hua et al. 2003), platelets, and other cell lines. Enhanced NADPH oxidase-generated ROS, which is regulated by protein kinase C (PKC), occurs in diabetic patients (Hink et al. 2001; Guzik et al. 2002; Quagliaro et al. 2003). H_2O_2, hydroxyl radical ($^{\bullet}OH$), and $^{\bullet}O_2^-$ are among the major by-products of xanthine oxidase, which is increased in diabetes mellitus (Butler et al. 2000; Desco et al. 2002). Uncoupled eNOS refers to the conditions in which constitutive eNOS does not have its substrate, L-arginine, or one of the five essential cofactor groups, namely, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH_4), and Ca^{2+}-calmodulin. Under such conditions $^{\bullet}O_2^-$ is produced instead of $NO^{\bullet}$. In addition, $NO^{\bullet}$ can react with superoxide to form peroxynitrite, which in turn oxidizes BH_4 and causes further uncoupling of NO formation (Yung et al. 2006). Involvement of lipoxygenase (LOX) products, particularly 12(S) and 15(S)-hydroxyeicosatetraenoic acids in the pathogenesis of diabetes-induced atherosclerosis, has been described. These two compounds facilitate the function of growth factors and proinflammatory cytokines (Dandona and Aljada 2002; Natarajan and Nadler 2003).

The cytochrome P450 monooxygenases are a large class of enzymes involved in the metabolism and detoxification of endogenous and exogenous materials. Dioxygen compounds, which decompose and release $^{\bullet}O_2^-$ and H_2O_2, are by-products of this process (Loida and Sligar 1993; Caro and Cederbaum 2004). Diabetes affects different isoforms of the cytochrome P450 system; for example, there is an increased expression of CYP2E1 in type 1 and 2 diabetic individuals (Haufroid et al. 2003; Wang et al. 2003) and *ob/ob* mice (genetically modified leptin-deficient obese mouse which is an animal model for type 2 diabetes) (Leclercq et al. 2000) and also in STZ-induced diabetic rats (Favreau et al. 1987). The upregulation of hepatic

CYP4A10 and CYP4A14 isoforms in *ob/ob* mice is thought to alleviate diabetes-induced hyperlipidemia since these enzymes are involved in fatty acid metabolism (Enriquez et al. 1999).

16.3 Antioxidant Therapy of Diabetes

There are two possible approaches for reduction of oxidative stress in diabetes: blocking formation of ROS/RNS radicals or boosting antioxidant defensive mechanisms. At the cellular level, it is feasible to inhibit several ROS-producing enzymes such as mitochondrial uncoupling proteins 1, NADPH oxidases, and inducible nitric oxide synthase (iNOS). However, due to the possibility of many adverse effects, human studies have used such interventions. Enhancing antioxidant system by antioxidant supplementation is the favorite approach tried by many investigators, with vitamins E and C, coenzyme Q10 (CoQ10), alpha-lipoic acid, and L-carnitine among the most commonly used antioxidants for the alleviation of diabetic complications.

16.3.1 Vitamin E

There are eight members in the vitamin E family, with four tocopherols (alpha, beta, gamma, and delta) and four tocotrienols (alpha, beta, gamma, and delta). Alpha-tocopherol is the most active form of this fat-soluble vitamin in humans. Vegetable oils, nuts, and green leafy produce are its main dietary sources; it is also available as a supplement. Vitamin E becomes oxidized while neutralizing free radicals and, in so doing, loses its antioxidant properties; however, other antioxidants such as CoQ10 and vitamin C reduce vitamin E and renovate its action. Vitamin E stimulates diacylglycerol kinase and inhibits phosphatidate phosphohydrolase in diabetic patients; the net effect of this is reduced diacylglycerol activity and consequent PKC activation; this is expected to impede the adverse effect of free fatty acids, which reduces skeletal muscle insulin sensitivity via PKC-dependent mechanisms. A substantial body of evidence derived from several controlled studies demonstrates the benefits of vitamin E supplementation in diabetic patients and in the general population (Stampfer et al. 1993; Knekt et al. 1994; Jialal and Fuller 1995; Lonn and Yusuf 1997). A double-blind crossover study reported that supplementation with 900 mg/day of vitamin E significantly reduced lipid, glucose, and HbA1c plasma levels, but it did not affect beta cell responsiveness to glucose in type 2 diabetic patients (Paolisso et al. 1993). In a prospective randomized case control study of 50 patients affected by type 2 diabetes, administration of 600 mg/day of vitamin E decreased HbA1c and insulin levels and improved cardiac autonomic regulation (Manzella et al. 2001). These effects, which are probably due to reduced oxidative stress, have been supported by other studies (Bonfigli et al. 2001; Gaede et al. 2001; Ruhe and

Table 16.1 Results of use of vitamin E effects in diabetes

• Reduction in protein kinase C activity
• Reduction of LDL oxidation
• Decreased platelet aggregation, enhanced vasodilation, and decreased plasminogen activator inhibitor type 1
• Decrease in urinary albumin excretion

McDonald 2001; Traber 2001; Park and Choi 2002; Huang et al. 2006). Vitamin E (680 IU daily) administered in combination with vitamin C (1250 mg daily) decreases urinary albumin excretion rate in patients with type 2 diabetes (Gaede et al. 2001).

In association with other antioxidant agents, vitamin E is also effective in treating diabetic neuropathy and retinopathy (Ruhe and McDonald 2001). Furthermore, alpha-tocopherol protects low-density lipoprotein (LDL) from oxidation, inhibits platelet aggregation, and enhances vasodilatation in diabetic patients (Traber 2001; Park and Choi 2002). Alpha-tocopherol also affects the expression and activity of immune and inflammatory cells and decreases plasminogen activator inhibitor type 1 (PAI-1), which are all thought to play important roles in diabetic vascular complications (Devaraj et al. 2002; Huang et al. 2006). Yet given this substantial body of evidence derived from several controlled studies demonstrating the benefits of vitamin E supplementation in reducing cardiovascular risk in the general population, the Heart Outcomes Prevention Evaluation (HOPE) Study was unable to show any significant benefits from the daily use of vitamin E in high-risk patients (Yusuf et al. 2000). In fact, a meta-analysis of 19 clinical trials, which included 135,967 subjects, concluded that high-dose vitamin E (16.5 to 2000 IU/day) could increase all-cause mortality. In the study of Suksomboon et al. (2011), vitamin E supplement did not show any benefit in controlling glycemic control of type 2 diabetic patients although it was effective in reducing HbA1c in those with low serum levels of vitamin E. Therefore, the possible usefulness of vitamin E supplementation in diabetics remains questionable, but its replenishment in individuals with low serum levels is reasonable. Table 16.1 summarizes some of the observed benefits of vitamin E in diabetic individuals (also see Table 16.6, which summarizes the findings from selected clinical trials with vitamin E in diabetic patients).

16.3.2 Vitamin C

Vitamin C (ascorbic acid) is a water-soluble vitamin with antioxidant activity. It is also able to recycle other important antioxidants from their radical forms, such as vitamin E and glutathione (Carr and Frei 1999). Decreased levels of vitamin C occur in diabetic patients, which make them prone to higher levels of lipid peroxidation (Sundaram et al. 1996; Maxwell et al. 1997). A highly significant inverse relation between plasma vitamin C and the risk of new-onset type 2 diabetes mellitus,

which is directly related to the amount of fruit and vegetable intake and diabetes, incidence has been reported in a population-based prospective cohort study of 21,831 healthy individuals over 12 years (Harding et al. 2008). There is also evidence for the interference by vitamin C in glucose metabolism. For instance, decreased levels of vitamin C during the glucose tolerance test have been reported in both diabetic and normal subjects (Ceriello et al. 1998). Meanwhile, supplementation with vitamin C in diabetic patient has shown beneficial effects in some studies. For example, chronic administration of 500 mg ascorbic acid twice a day improved fasting blood glucose and decreased HbA1c (Paolisso et al. 1995). There is evidence that ascorbic acid might directly improve insulin action, glycemic control, and endothelial function (Paolisso et al. 1994; Regensteiner et al. 2003). Vitamin C is also effective in reducing hypoglycemia-induced oxidative stress. In a study of patients with type 1 diabetes, vitamin C not only showed protective effects in the generation of oxidative radicals following hypoglycemia but also augmented the beneficial effects of glucagon-like peptide 1 (Ceriello et al. 2013).

Some prospective studies also showed that insufficient vitamin C intake is associated with greater cardiovascular risk (Osganian et al. 2003). However, the results of vitamin C supplementation in reducing cardiovascular complications do not support this contention. A prospective study by Osqanian et al. of more than 85,000 women over 16 years reported that users of vitamin C supplements appeared to be at a lower risk for coronary heart disease (Osganian et al. 2003). However, vitamin C supplementation in well-nourished populations fails to provide any additional benefit (Kushi et al. 1996; Losonczy et al. 1996). There was also no significant reduction in the 5-year mortality from, or incidence of, any type of vascular disease, cancer, or other major outcomes in high-risk individuals following antioxidant supplementation (Heart Protection Study Collaborative Group 2002). Genetic variations may confound studies of vitamin C supplementation in cardiovascular disease. For instance, reanalyzing the results of a randomized controlled trial using vitamin C combined with vitamin E and relating its effects to the haptoglobin genotype revealed that the usefulness of vitamin therapy on the progression of coronary artery stenosis in postmenopausal women depended on the haptoglobin type (Levy et al. 2004). Even though the importance of this observation is currently unclear, it represents an area that warrens further detailed investigations so that we can better understand some of the opposing results of vitamin C administration in diabetic patients. Table 16.7 depicts some of the clinical trials with vitamin C in diabetic patients.

16.3.3 Alpha-Lipoic Acid

Alpha-lipoic acid (ALA), which is also known as 1,2-dithiolane-3-pentanoic acid or thioctic acid, is a natural product that is synthesized in many plants and human body from octanoic acid and cysteine (Reed 1998). ALA acts as a cofactor for pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase in humans (Schmidt et al. 1994) and also has a critical role in oxidative decarboxylation of pyruvate to

acetyl-CoA (Reed 1998). There are two optical isomers (R and S forms) for lipoic acid; while the R isomer is the natural form of this substance and act as an important cofactor in biological system, S isoform impedes R polymerization and improves its bioavailability (Shay et al. 2009). ALA is reduced in mitochondria where an NADH-dependent reaction with lipoamide dehydrogenase converts it to dihydrolipoic acid (DHLA). Both ALA and DHLA possess an impressive array of antioxidant activities including (1) quenching of ROS, (2) regeneration of exogenous and endogenous antioxidants such as vitamin C, vitamin E, and glutathione, (3) chelation of metal ions, (4) reparation of oxidized proteins, and (5) regulation of gene transcription and inhibition of nuclear factor kappa B (NFκB) activation (Biewenga et al. 1997). ALA is not only synthesized by the liver but also can be acquired from different nutritional resources such as spinach, broccoli, tomato, garden peas, brussel sprouts, rice bran, liver, kidney, and heart (Packer et al. 2001). ALA can also be obtained as a supplement; however, ALA in foods is attached to proteins (lipoyllysine), while ALA in supplements is unbound (Singh and Jialal 2008).

ALA has beneficial effects in diabetes in multiple ways. First, it activates 5′ AMP-activated protein kinase (AMPK), which is considered a fuel sensor in cells. A variety of cellular stresses (hypoxia, hypoglycemia, oxidative stress, etc.) lead to decreased adenosine triphosphate (ATP) and/or increased adenosine diphosphate (ADP)/adenosine monophosphate (AMP) levels; these changes activate AMPK, which then inhibits energy-consuming pathways (Kola et al. 2006). Activation of AMPK in hepatocytes reduces gluconeogenesis by downregulation of the expression of genes involved in glucose metabolism (e.g., phosphoenolpyruvate carboxykinase and glucose-6-phosphatase), so resulting in decreased glucose output to the circulation (Viollet et al. 2009). Importantly AMPK activation in skeletal muscles increases glucose uptake and fatty acid oxidation through overexpression of glucose transporter 4 (GLUT4) and its translocation to the cell membrane (Konrad et al. 2001). AMPK activation also improves insulin sensitivity by phosphorylating and inactivating acetyl-CoA, which reduces malonyl-coenzyme A (Ruderman and Flier 2001). Reduction of malonyl-CoA results in decreased synthesis and increased oxidation of fatty acids, which consequently decreases skeletal muscle triglycerides content (Winder and Hardie 1996). Accumulation of triglycerides in skeletal muscles is an important factor for insulin resistance in obesity and diabetes (Goodpaster and Kelley 2002). ALA increases the activity of AMPK and fatty acid oxidation in Obese Long Evans Tokushima Fatty rats, which are normally deficient in muscular AMPK and prone to diabetes (Lee et al. 2005a, b). ALA also enhances whole-body insulin-dependent glucose consumption (Lee et al. 2005a, b). Overexpression of AMPK in normal mice is associated with mild hypoglycemia (Foretz et al. 2005) and in diabetic mice prevents hyperglycemia (Viana et al. 2006). Recent data implicating a role for AMPK in the hypoglycemic effects of the antidiabetic drugs metformin and thiazolidinedione has added a perspective to possible therapeutic manipulation of this enzyme (Musi et al. 2002; Saha et al. 2004). AMPK is also expressed in the hypothalamus, which has a critical role in adjusting appetite and satiety. Increased hypothalamic AMPK activity results in augmentation of appetite and food consumption (Andersson et al. 2004). On the other hand, leptin, insulin,

glucose, and ALA all reduce the activity of hypothalamic AMPK and reduce food consumption (Lee et al. 2005a, b). Intraventricular administration of AICAR (5-aminoimidazole-4-carboxamide ribotide), an AMPK activator, reverses the effect of ALA on appetite (Kim et al. 2004), suggesting that the anti-obesity effect of ALA is mediated through inhibition of hypothalamic AMPK. Administration of ALA (1800 mg/day, 20 weeks) to diabetic patients resulted in modest weight reduction (Koh et al. 2011).

Another beneficial effect of ALA occurs via modification of peroxisome proliferator-activated receptors (PPAR)-regulated genes. PPARs are a group of nuclear proteins, which adjust gene transcription and have multifaceted roles in critical cell processes such as cellular differentiation, tumorigenesis, and metabolism (Berger and Moller 2002; Michalik et al. 2006; Belfiore et al. 2009). For instance, PPAR-α impacts the expression of carnitine palmitoyltransferase 1A and acetyl-CoA synthase. PPAR-γ promotes the expression of fatty acid translocase/CD36, adipocyte fatty acid-binding protein, and lipoprotein lipase (Pershadsingh 2007). These enzymes have an essential role in energy metabolism. ALA activates both PPAR-α and PPAR-γ (Butler et al. 2009; McCarty et al. 2009). Overexpression of PPAR α in skeletal muscles improved mitochondrial respiration and promoted GLUT4 transcription (Michael et al. 2001). Wang et al. (2010) showed that ALA supplementation in mice increased expression of PPAR coactivator-1α. There is also some evidence for the beneficial effects of ALA on hyperglycemia-induced reduction of PPAR-γ, hyperinsulinemia, insulin resistance, systolic hypertension, and superoxide production (Midaoui et al. 2003; El Midaoui et al. 2006).

ALA also exerts its beneficial effects in diabetes through pancreatic beta cell protection; however, this action seems to be dose dependent. It has been reported that both acute and chronic administration of ALA to beta cell cultures increased ROS production and decreased insulin secretion (Targonsky et al. 2006). Likewise, AMPK overactivity in adenovirus-treated beta cells reduced insulin secretion, deterioration of glycemic control, and a higher apoptotic index (Richards et al. 2005). Considering the opposing effects of ALA at lower and higher concentrations could potentially be a response to this paradox. For example, ALA has both antiproliferative and proliferative effects in tumor cells at 1 and 100 μmol/l, respectively (Dovinová et al. 1999). Concentrations of ALA that activate AMPK in beta cells (500 μmol/l) (Targonsky et al. 2006) are significantly higher than those with ALA during treatment of diabetic neuropathy (Niebch et al. 1997; Chen et al. 2005). Lee et al. (2009) investigated the dose-dependent effect of ALA on rat insulinoma cells. Higher concentrations (>300 μmol/l) induced apoptosis, while reductions in hydrogen peroxide-induced apoptosis and ROS production, mitochondrial membrane depolarization, and c-JNK activation required lower concentrations (150–300 μmol/l). It seems reasonable to suggest that therapeutic concentrations of ALA have cytoprotective effects on pancreatic beta cells exposed to high levels of oxidative stress (e.g., diabetes), while supraphysiologic levels can be harmful and adversely affect the cell function.

One potential mechanism involved in the pathogenesis of diabetic complications, specially nephropathy and retinopathy, involves protein glycation (Brownlee

et al. 1988; Schalkwijk et al. 2002). Inhibition of glycation might be another mechanism for the beneficial effects of ALA (Suzuki et al. 1992; Kawabata and Packer 1994). It is assumed that ALA and DHLA interact with the active sites on albumin molecules through hydrophobic binding (Kawabata and Packer 1994), and this prevents the nonenzymatic reaction between glucose and plasma proteins and the development of Amadori products.

The role and mechanisms of redox imbalance in diabetic vasculopathy have been described elsewhere (Golbidi et al. 2012). In brief, diabetes-induced oxidative stress prompts a proinflammatory state, which through a series of reactions impairs NO•-mediated vasodilation. Activation of PKC and NFκB decreases the expression of eNOS and increases the expressions of endothelin, vascular endothelial growth factor, plasminogen activator inhibitor-1, transforming growth factor-β, NADPH oxidases, and several other inflammatory mediators (Sena et al. 2008). ALA restores endothelial function and improves systemic and local oxidative stress (Sena et al. 2008). This has been shown in both animal and human studies; for instance, the effects of lipoic acid (0.2 mM) on forearm blood flow were evaluated in response to acetylcholine, sodium nitroprusside, and after concomitant infusion of the NO•-inhibitor and N(G)-monomethyl-L-arginine in diabetic and normal individuals. This experiment confirmed the beneficial effects of ALA, particularly in patients with impaired redox status (Heitzer et al. 2001). It is likely that ALA restores eNOS function partially through enhancement of its phosphorylation by Akt (Smith and Hagen 2003). Insulin receptor tyrosine kinase, phosphatidylinositol 3-kinase (PI 3-kinase), and Akt are essential components of insulin signaling pathways related to endothelial cell production of NO• (Montagnani et al. 2002). ALA also increases NO• bioavailability in human umbilical endothelial cells and monocyte cells by induction of Akt phosphorylation, with potential implications for the prevention of sepsis and inflammatory vascular diseases (Artwohl et al. 2007; Zhang et al. 2007). Therefore, it can be concluded that ALA improves endothelial function at least partially by recoupling of eNOS.

Additionally, ALA decreases the amount of asymmetric dimethylarginine (ADMA). ADMA is an endogenous product resulted from methylation of arginine via N-methyltransferase and acts as a circulating NOS inhibitor. This inhibitory effect can be reversed by adequate amounts of L-arginine (Boger RH et al. 1998). The presence of ADMA has been reported in human plasma and urine (Vallance et al. 1992; Böger 2003). Physiologic levels of L-arginine provide adequate substrate for NOS and NO production in healthy individuals. Increased levels of ADMA have been reported in many pathophysiological conditions including diabetes, hypertension, preeclampsia, congestive heart failure, and chronic renal failure (Pettersson et al. 1998; Vallance 2001; Zoccali et al. 2001; Lin et al. 2002). ADMA is excreted by the kidney but is largely metabolized by the oxidative stress sensitive enzyme dimethylarginine dimethylaminohydrolase (DDAH) (Mittermayer et al. 2007). DDAH is widely distributed throughout the body including kidneys, pancreas, brain, liver, lungs, endothelium, and, myocardium (Nijveldt et al. 2003a, b). Decreased activity of DDAH, resulting in increased levels of ADMA, occurs in hypercholesterolemia and diabetes (Ito et al. 1999; Lin et al. 2002). An increased

Table 16.2 Rationale for using ALA in diabetes

• Activation of 5′ AMP-activated protein kinase
– Inhibition of energy-consuming pathways
– Reduction of gluconeogenesis
– Increased glucose uptake and fatty acid oxidation in skeletal muscles
– Increased peripheral sensitivity to insulin
• Modification of peroxisome proliferator-activated receptors-regulated genes
– Improved mitochondrial respiration and glucose consumption in skeletal muscles
• Cytoprotective effects on pancreatic beta cells
• Inhibition of glycation
• Restoration of endothelial cell function
• Improvement in local and systemic oxidative stress

concentration of ADMA suggests cardiovascular complications. There are several reports confirming ADMA-induced endothelial dysfunction, increased vascular resistance, and decreased cardiac output in different patient populations including diabetic individuals (Calver et al. 1993; Achan et al. 2003; Tarnow et al. 2004). It seems that ADMA is not only a risk marker for cardiovascular adverse outcomes but may also participate as an etiologic factor (Krzyzanowska et al. 2008). ALA could possibly reduce ADMA levels by protecting the redox-sensitive enzyme DDAH (Mittermayer et al. 2007). ALA also increases the expression of DDAH and amplifies signal transducer and activator of transcription 3 (STAT3) transfection-induced increase in DDAH II promoter activity (Lee et al. 2010). Table 16.2 depicts important rationale for using ALA in diabetic patients (also see Table 16.8, which demonstrates some of the clinical trials with ALA in diabetic patients).

16.3.4 L-Carnitine

Carnitine, a L-β-hydroxy-γ-N-trimethylaminobutyric acid, is a water-soluble substance that is essential for the metabolism of lipids (Rebouche and Seim 1998). It can be biosynthesized from the amino acids lysine and methionine in the liver and kidneys or obtained from animal dietary sources such as meat, poultry, or dairy products. In cases of strict vegetarianism, endogenous biosynthesis provides 90% of daily requirements. On the other hand, carnitine supplementation above daily needs suppresses internal biosynthesis. The availability of several cofactors such as vitamin C, vitamin B6, and iron is essential for carnitine synthesis. A deficiency of these micronutrients leads to carnitine deficiency particularly when there is shortage from dietary sources (Rebouche et al. 1989). The function of organic cation transporters (OCTNs), a family of proteins responsible for transporting cations across the cell membrane, is critical for carnitine uptake in tissues incapable of carnitine synthesis (Scaglia et al. 1999). OCTNs also have an essential role in maintaining serum carnitine levels, as they regulate carnitine reabsorption in renal tubules. Mutations in the OCTN gene lead to carnitine deficiency secondary to urinary loss (Scaglia et al. 1999). Carnitine has two stereoisomers: while L-carnitine is the bioactive form, the D isomer is inert. L-Carnitine facilitates the transport of fatty acids through the mitochondrial membrane for

β-oxidation. In addition, carnitine carries acetyl groups across the mitochondrial membrane and therefore has an essential role in the metabolism of glucose and assists in cellular energy level (Johri et al. 2014). Impaired transport of fatty acids across mitochondrial membrane leads to triglycerides accumulation in the cytosol and is an important factor in the pathogenesis of atherosclerosis and insulin resistance (Bartel et al. 1982; Krajcovicová-Kudlácková et al. 2000). An antihyperlipidemic effect of carnitine has been shown in several animal studies; for instance, carnitine treatment of rabbits fed a high-fat diet reduced plasma lipids (triglycerides, very low-density lipoprotein (VLDL), and intermediate-density lipoprotein (IDL) cholesterol), the extent and thickness of atherosclerotic plaques, and lowered the percentage of macrophage-derived cells inside atheromatous plaques (Spagnoli et al. 1995). In a similar experiment where atherosclerosis was induced by a high-fat diet in rabbits, hypocarnitinemia, induced by administration of D-carnitine for 28 days, aggravated this process, whereas L-carnitine prevented it (Sayed-Ahmed et al. 2001). Diaz Gómez et al. (2006) also confirmed the favorable effects of L-carnitine on total cholesterol and cholesterol lipoproteins in rabbits. Infusion of carnitine to young healthy volunteers resulted in 50% increase in non-oxidative glucose removal that was associated with decreased levels of circulating free fatty acids, glycerol, and beta-hydroxybutyrate (Ferrannini et al. 1988). Acute administration of carnitine to diabetic patients increased whole-body glucose utilization that was accompanied by decreased levels of lactate and free fatty acids (Capaldo et al. 1991). Chronic administration of oral carnitine increases glucose disposal rate, improves blood pressure, and ameliorates hypoadiponectinemia (Ruggenenti et al. 2009). In a multicenter study in Europe, propionyl-L-carnitine (2 g/day, 12 months) increased both maximal walking distance and initial claudication distance in patients with peripheral vascular disease. This effect was more prominent in those who had significant functional impairment (Brevetti et al. 1999). Acute administration of propionyl carnitine (600 mg in 100 ml of normal saline, twice a day/10 days) in diabetic patients with peripheral vascular disease also improved maximal walking distance, initial claudication distance, and glycemic control (Ragozzino et al. 2004). In another study, where amelioration of diabetic neuropathy was the targeted outcome, acetyl-L-carnitine (1000 mg/day, for 10 days, followed by 2000 mg/day up to 1 year) was effective and well tolerated (De Grandis and Minardi 2002).

There are several promising reports on the beneficial effects of L-carnitine in reducing insulin resistance, dyslipidemia, atherosclerosis, obesity, metabolic syndrome, and hypertension, albeit with some controversy. This emphasizes on the need for larger, well-designed clinical trials, which could clarify the clinical criteria for routine prescription of this substance in diabetic patients and also those with other cardiovascular diseases. Table 16.3 shows some findings on the use of L-carnitine in diabetic patients (also see Table 16.9, which describes data from the clinical trials with carnitine in diabetic patients).

Table 16.3 Important benefits of using L-carnitine in diabetic patients

• Facilitation of fatty acid transport across mitochondrial membrane
• Improved glucose metabolism by transporting acetyl group across mitochondrial membrane

16.3.5 Coenzyme Q10

CoQ10, also known as vitamin Q10, vitamin-like quinone, or ubiquinone, is a fat-soluble substance. Originally known for its effect on mitochondrial respiratory chain and oxidative phosphorylation, its presence has since been recognized in the plasma and other subcellular components (Littarru and Tiano 2010). The highest concentration of CoQ10 occurs in the tissues with high-energy turnover such as the heart, liver, kidneys, and pancreas. Since the human body is able to synthesize CoQ10, it is not considered an essential nutrient. There are three major stages in CoQ10 synthesis: the first step is synthesis of the ring structure from tyrosine or phenylalanine, this is followed by the formation of side chain from acetyl-CoA, and the final step involves the incorporation of these structures by the enzyme polyprenyltransferase (Overvad et al. 1999). Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, a critical enzyme in cholesterol biosynthesis, also has an important role in CoQ10's side-chain synthesis (Hargreaves et al. 2005). Ubiquinone is found in many food products, including beef, poultry, or broccoli, and can be supplied exogenously. CoQ10 deficiency results from reduced endogenous synthesis or an increase in daily demand. Decreased production occurs secondary to a limited supply of amino acids (phenylalanine) or a reduction of cholesterol biosynthesis. Although there is not an established link between statin use and decreased tissue CoQ10 content, some studies relate the adverse effects of these medications (myopathy) to lower levels of CoQ10 (Hargreaves et al. 2005; Marcoff and Thompson 2007). An increased need for CoQ10 has been reported in the elderly and in several pathophysiologic states including heart failure and diabetes (Langsjoen and Langsjoen 1999).

CoQ10 functions as an electron transporter during cellular respiration; it receives electrons from a variety of donors such as NADH, succinate hydrogenase, or glycerol-3-phosphate and conveys them to the cytochrome system (Crane and Navas 1997). This action provides a proton gradient inside the inner mitochondrial membrane, which is then used for ATP synthesis (Tran et al. 2001). The antioxidant effects of CoQ10 depend on reduction to ubiquinol (Littarru and Tiano 2010). CoQ10 can reduce lipid peroxidation and protect other antioxidant radicals, such as vitamin E, from oxidation (Alleva et al. 1995). There is some support for the suggestion that CoQ10 not only prevents but also halts the propagation of lipid and protein oxidation (Bentinger et al. 2010). Other functions for CoQ10 are thought to include cell membrane stabilization through direct interaction with membrane phospholipids, inhibition of intracellular phospholipases, preservation of myocardial Na-K ATPase activity, and regulation of prostaglandin metabolism (Greenberg and Frishman 1990).

The wide-ranging biological properties of CoQ10 increase the potential for its clinical application; however, in the case of CoQ10 (100 mg/day/6 month), it did not show any metabolic improvement in terms of glycemic control or lipid profile in type 2 diabetes (Eriksson et al. 1999), while a 3-month treatment with CoQ10 did

Table 16.4 Potential benefits of CoQ10 in diabetic patients

• Reduction in lipid peroxidation
• Protecting other antioxidant radicals such as vitamin E
• Stabilization of cell membrane
• Inhibition of intracellular phospholipases
• Regulation of prostaglandin metabolism

not decrease insulin requirements in patients with type 1 diabetes (Henriksen et al. 1999). Of importance is that CoQ10 can improve endothelial function. For example, a double-blind, randomized, controlled trial reported that administration of CoQ10 (300 mg/day) augmented extracellular superoxide dismutase (SOD) levels and improved endothelial-dependent vasodilation in patients with coronary artery disease. This effect was more prominent in patients who initially had low levels of SOD and were thus prone to higher degrees of oxidative stress (Tiano et al. 2007). In another double-blind placebo-controlled crossover study by Belardinelli et al., 23 patients with stable congestive heart failure secondary to ischemic heart disease were treated with CoQ10 300 mg/day for 1 month. Treatment with vitamin Q10 improved functional capacity, endothelial function, and left ventricular contractility. Data from another cohort sample of 236 patients with congestive heart failure suggests that the plasma concentration of CoQ10 was an independent risk factor of mortality (Molyneux et al. 2008). Although there are no clear benefits for using CoQ10 in diabetes, its effects on improving lipid profile and cardiac and endothelial function make it a good candidate worthy of further evaluation. Table 16.4 summarizes potential benefits of CoQ10 in diabetic patients (also see Table 16.10, which summarizes data from clinical trials with CoQ10 in diabetic patients).

16.3.6 Ruboxistaurin

The splendid insight by Brownlee led to our current understanding that mechanisms of diabetes-induced oxidative stress and its subsequent vascular consequences can be summarized according to four main pathophysiologic pathways (Brownlee 2001), namely, the polyol, hexosamine, advanced glycation end product, and PKC pathways. Theoretically, inhibiting any of these pathways should result in reducing some of the adverse effects of diabetes. Ruboxistaurin, a specific PKC-β isoform inhibitor, has shown promising effects in ameliorating some diabetic complications (Alicic and Tuttle 2014).

PKC is an intracellular signal transducer with at least 13 isoenzymes and which can activate a series of secondary kinases (Das Evcimen and King 2007). Activation of this chain reaction potentially modifies cell behavior in terms of metabolism, surface proteins, and gene transcription (Pathak et al. 2012). The activation of different isoforms of PKC probably depends on substrate specificity,

intracellular location, or preferential cellular expression (Danis and Sheetz 2009). There is ample of evidence confirming overactivity of PKC-β in various tissues during the course of diabetes (Keranen et al. 1995; Mellor and Parker 1998; Das Evcimen and King 2007). Increases in the cellular content of glucose during hyperglycemic states lead to amplification of diacylglycerol (DAG) production, which is one of the most important activators of PKC (Clarke and Dodson 2007). Activation of PKC in vasculature leads to increased production of extracellular matrix proteins and cytokines, alteration in vascular contractility/permeability, changes in cellular proliferation, increased activity of cytosolic phospholipase A2, and reduced Na+/K+ ATPase activity (Golbidi et al. 2011). These functional and structural alterations result in the pathogenesis of diabetes-induced vasculopathy. For instance, increased expression of endothelin-1 (ET-1) following PKC activation results in enhanced activation of mitogen-activated protein kinase (MAPK) in mesangial cells, which is an important mechanism in the pathogenesis of diabetic glomerular disease (Glogowski et al. 1999). Heightened expression of vascular endothelial growth factor (VEGF) and increased vessel permeability are other consequences of PKC activation (Williams et al. 1997). Decreased expression of eNOS (Kuboki et al. 2000), overexpression of PAI-1 (Feener et al. 1996), and amplified expression of transforming growth factor-β, fibronectin, and collagen (Koya et al. 1997) are other pathways that also negatively affect the vascular system in diabetes.

Neuropathy is one the most common and also possibly the most bothersome consequence of diabetes. It can affect both the somatic and autonomic nervous system. Affliction of the autonomic nervous system induces a series of cumbersome symptoms such as orthostatic hypotension, tachycardia, urinary retention, and gastroparesis among other symptoms. The involvement of the peripheral nervous system usually starts with demyelination of small C fibers and manifests as impairment in vibratory perception, pain, burning, tingling, and numbness of the feet and lower legs (Danis and Sheetz 2009). Vascular microangiopathy, which is partially responsible for the pathophysiology of diabetic neuropathy (as proper nerve function depends on an adequate blood supply), histologically manifests as thickening of the capillary basement membrane and endothelial hyperplasia (Danis and Sheetz 2009). In a study in animals 8 weeks after the induction of diabetes, Cotter et al. (2002) demonstrated decreases in sciatic motor and saphenous nerve sensory conduction velocity, mechanical and thermal hyperalgesia, impairment of sciatic nerve and superior cervical ganglion blood flow, and decreased vasodilation of superior mesenteric artery in response to acetylcholine. Treatment with ruboxistaurin (LY33353) suppressed all these complications except mechanical hyperalgesia (Cotter et al. 2002). However, in spite of the promising effects shown in this and other experiments, ruboxistaurin is unable to cause similar benefits in a multinational, randomized, double-blind, placebo-controlled human study (Vinik et al. 2005). In this study the therapeutic effects of 32 and 64 mg/day of ruboxistau-

rin was compared with placebo in 205 patients that were matched based on demographic and baseline specifications and enrolled in one of the three treatment arms (32 mg/day, 64 mg/day, and placebo) for 1-year trial. Overall, there were no significant differences between the groups with regard to indicators of diabetic peripheral neuropathy in terms of vibration detection threshold and neuropathy total symptoms score, except in a subgroup of patients with less severe symptoms.

Ruboxistaurin has also been used in the treatment of diabetic retinopathy. Following promising results from phase I (Demolle et al. 1998, 1999) and phase II (Aiello et al. 2006) clinical trials, two large phase III randomized controlled multicenter trials were conducted between 1998 and 2002 (PKC-DRS Study Group 2005, 2007). The PKC-Diabetic Retinopathy Study (PKC-DRS) examined the safety and efficacy of three daily doses of ruboxistaurin and compared these with placebo in 252 patients who were followed for 36–48 months. Only the treatment with 32 mg/day was able to delay sustained visual loss (PKC-DRS Study Group 2005). The PKC-Diabetic Macular Edema Study (DMES) enrolled 686 individuals and also evaluated the effects of three doses (4, 16, 32 mg/day) of ruboxistaurin for at least 30 months on macular edema (PKC-DRS Study Group 2007). The primary outcome was progression to sight-threatening macular edema or application of focal/grid photocoagulation of diabetic macular edema. Even though no significant effects were observed with ruboxistaurin in terms of primary outcome measures, the 32 mg dose delayed progression of diabetic macular edema to a sight-threatening stage (PKC-DRS Study Group 2007). Another randomized, double-blind placebo-controlled multicenter trial (PKC-DRS2) also reported favorable results for treatment with 32 mg/day with ruboxistaurin for the treatment of diabetic retinopathy in terms of vision loss, need for laser therapy, and macular edema (PKC-DRS2 Group 2006). Based on the encouraging results with ruboxistaurin, the Eli Lilly Company received an "approvable" letter from the FDA in 2006, but requesting for additional phase III clinical evidence (Danis and Sheetz 2009). Table 16.5 discusses some advantages of inhibiting the PKC pathway in diabetic patients (also see Table 16.11, which lists findings from clinical trials using ruboxistaurin in diabetic patients).

Table 16.5 Potential benefits of ruboxistaurin in diabetic patients

• Inhibition of protein kinase C pathway which potentially leads to:
– Change in extracellular matrix proteins and cytokines
– Improved vascular permeability
– Reduction of endothelin-1 expression
– Decreased vascular endothelial growth factor expression
– Improved eNOS expression
– Decreased plasminogen activator inhibitor 1

Table 16.6 Selected clinical trials with vitamin E in diabetic patients (source Golbidi et al. Curr Diabetes Rev. Golbidi et al. 2011, with permission)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Outcome	Results
Kataja-Tuomola et al. (2010)	29,133 male smokers (1700 T2D)	Vitamin E (50 mg/day) β-Carotene (20 mg/day) Vitamin E + β-carotene Placebo	6.1 years	19 years	Macrovascular complications Total mortality	No effect
Milman et al. (2008)	1434 with DM and low Haptoglobin level (Hp 2–2 genotype)	Vitamin E (400 U/day) Placebo	18 months		MI, stroke, cerebrovascular death	Effective
Kataja-Tuomola et al. (2008)	29,133 male smokers	Vitamin E (50 mg/day) β-Carotene (20 mg/day) Vitamin E + β-carotene Placebo	6.1 years	12.5 years	Prevention of T2D	No effect
Winterbone et al. (2007)	19 T2D	1200 IU/day Placebo	4 weeks		Oxidative DNA damage	Increased damage after acute hyperglycemia
Wu et al. (2007)	55 T2D	αT (500 mg/day) Mixed (αT and γT) Placebo	6 weeks		Cellular T level Plasma and urine F(2)-isoprostanes RBC antioxidant activity Plasma inflammatory marker	↑ cellular T level with mixed T ↓ plasma F2 isoprostanes
Ward et al. (2007)	58 T2D	αT (500 mg/day) Mixed (αT and γT) Placebo	6 weeks		24-h ambulatory BP, HR Plasma and urine F(2)-isoprostanes Endothelium-dependent and endothelium-independent vasodilation	↑ BP, HR, and pulse pressure with both αT and γT ↓ plasma F2 isoprostanes – No effect on vasodilation

Clarke et al. (2006)	58 T2D	αT (500 mg/day) Mixed (αT and γT) Placebo	6 weeks	Serum and cellular T concentration Urinary T metabolite excretion In vivo platelet activation	↑ in serum and cellular γT with γT treatment No effect on markers of platelet activation
Ble-Castillo et al. (2005)	34 T2D	αT (800 IU/day) Placebo	6 weeks	Malondialdehyde HbA_{1c} GPX, SOD (in RBCs) Glucose and lipid	↓ RBC malondialdehyde ↑ cholesterol levels ↓ SOD No benefit on glycemic or lipid control
Manuel-Y-Keenoy et al. (2004)	22 T1D with high cholesterol	Atorvastatin (20 mg) + αT (750 IU) Atorvastatin (20 mg)	6 months	Blood chemistry Lipoprotein subfractions Lipid peroxidation	No additional effect in lowering plasma lipids or malondialdehyde from vitamin E
Park and Choi (2002)	98 T2D	Continuous S.C. insulin infusion (CSII) plus 200 mg αT CSII plus placebo	2 months	Lipid peroxidation in plasma and RBC	Vitamin E decreased blood lipid peroxidation without altering antioxidant enzyme activity
Devaraj et al. (2002)	47 T2D	Vitamin E 1200 IU/day	3 months	Plasminogen activator inhibitor-1 P-Selectin	Vitamin E decreased markers of thrombosis in diabetic and controls
Gaede et al. (2001)	30 T2D	Vitamin E (680 IU/day) + vitamin C (1250 mg/day)	4 weeks	Albuminuria Serum Cr, Hb A_{1c}, blood pressure	Decrease albuminuria No change in serum Cr, Hb A_{1c}, blood pressure

BP blood pressure, *Cr* creatinine, *HR* heart rate, *MI* myocardial infarction, *RBC* red blood cells, *SOD* superoxide dismutase, *GPX* glutathione peroxidase, *T* tocopherol

Table 16.7 Selected clinical trials with vitamin C in diabetic patients (source Golbidi et al. 2011, with permission)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
Holmes et al. (2010)	762 pregnant women with T1D	Vitamin C (1000 mg/day) + vitamin E (400 IU/day) Placebo	Between 8 and 22 weeks of pregnancy until delivery		Incidence of preeclampsia	No benefit
Song et al. (2009)	8171 women (health professionals) with CVD or ≥3 risk factors for CVD	Vitamin C (500 mg/day) Vitamin E (600 IU/every other day) β-Carotene (50 mg/every other day)	~9.2 years	~9.2 years	Primary prevention of T2D	No benefit
Hercberg et al. (2009)	3146 middle-age people	Combination of vitamin C (120 mg/day), vitamin E (30 mg/day), β-carotene (6 mg/day), zinc (15 mg/day), selenium (100 μg/day) Placebo	~ 7.5 years	~ 7.5 years	Primary prevention of cardiovascular disease (diabetes)	No benefit
Gutierrez et al. (2009)	12 patient with controlled T2D	No vitamins αT [(200 IU) + vitamin C (250 mg)]/day αT [(400 IU) + vitamin C (500 mg)/day αT [(800 IU) + vitamin C (1000 mg)/day	2 weeks		Surrogate markers of oxidative stress (oxy-LDL, MDA, NEFAs, RBC's GPX) Surrogate markers of inflammation (CRP, adiponectin, IL-6) Surrogate markers of hypercoagulation (PAI-1, fibrinogen activity)	No benefit

Chui and Greenwood (2008)	16 patients with T2D	High-fat diet plus supplemental vitamin E (800 IU) and vitamin C (1000 mg) High-fat diet without vitamins Water	3 test sessions separated by 1 week		Postprandial memory impairment	Antioxidant vitamins could prevent meal-induced memory impairment
Rizzo et al. (2008)	13 older men with IFG	Vitamin E (1000 mg/day) + vitamin C (1000 IU/day)	4 weeks		WBGD Plasma levels of antioxidant and inflammatory cytokines	Increase in WBGD Reduced inflammation Improved insulin sensitivity
Davison et al. (2008)	12 patients with T1D and 14 controls	Vitamin C (1000 mg) Placebo			Assessment of exercise-induced oxidative stress (measured by PBN adduct extraction and EPR analysis + markers of lipid peroxidation)	Vitamin C had prophylaxis effect against exercise-induced ROS production
Harding et al. (2008)	21,831 healthy individuals	Daily fruit and vegetables consumption		12 years	Incidence of diabetes	Strong inverse association between plasma vitamin C and diabetes
Afkhami-Ardekani and Shojaoddiny-Ardekani (2007)	84 patients with T2D	Vitamin C (500 mg/day) Vitamin C (1 g/day)	6 weeks		Metabolic control (FBS, TG, TC, LDL, HDL, Hb_{1c})	Improved metabolic control with 1 g/day vitamin C

(continued)

Table 16.7 (continued)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
Tessier et al. (2009)	36 elderly patients with T2D	Vitamin C (500 mg/day) Vitamin C (1 g/day) Placebo	12 weeks		Intracellular level of vitamin C and glutathione Lipid peroxidation markers Vitamin E content of LDL LDL susceptibility to peroxidation	Increased cellular glutathione with both doses Increased vitamin C and E content of LDL with 1 g/day dose No change in lipid peroxidation markers and LDL susceptibility to peroxidation
Waring et al. (2006)	8 men with T1D 8 regular smokers 8 healthy controls	1 g uric acid i.v. 1 g vitamin C i.v. Vehicle 0.9% saline			Forearm blood flow response to Ach and SNP	Vitamin C (and uric acid) improved response to Ach (but not to SNP)
Tousoulis et al. (2007)	41 patients with T2D	Vitamin C (2 g/day) Atorvastatin (10 mg/day) No treatment	4 weeks		FBF Forearm vasodilatory response to postischemic hyperemia (RH %) Endothelium-independent vasodilation (NTG %) TC, HDL, TG, BS, CRP sVCAM-1, TNF-α, IL-6, ADMA	Atorvastatin ↓ IL-6, TNF-α, sVCAM, CRP, and ADMA and ↑ maximum hyperemic FBF, RH% Vitamin C had no effect on these parameters

Neri et al. (2005)	46 patients with T2D 46 patients with IGT 46 healthy controls	A standard diet for 10 days followed by 15 days standard diet plus standard antioxidant treatment (N-acetylcysteine 600 g/day, vitamin E 300 g/day, vitamin C 250 mg/day) in all groups		Comparison of LDL, malondialdehyde, NO, endothelin-1, vWF, VCAM-1, BS, TG, and HbA_{1c}, before and after antioxidant therapy	Antioxidant decreased oxidative stress in all groups
Chen et al. (2006)	32 selected T2D with low vitamin C level	Vitamin C (800 mg/day) Placebo	4 weeks	Insulin sensitivity Forearm blood flow in response to Ach, SNP, or insulin	No change in FBS, insulin sensitivity, or blood flow response to Ach or SNP
Lu et al. (2005)	17 patients with T2D	Vitamin C (1 g/day) Placebo	2 weeks	Microvascular reactivity (assessed by vital capillaroscopy and PRH (post-occlusive reactive hyperemia) hsCRP, IL-6, IL-1ra, ox-LDL	No significant change
Farvid et al. (2004a, b)	69 patients with T2D	Mg (200 mg/day) + Zn (30 mg/day) Vitamin C (200 mg/day) + vitamin E (150 mg/day) Minerals plus vitamins (above doses) Placebo	3 months	Systolic, diastolic, and mean arterial BP TG, TC, HDL, LDL, apolipoprotein A1 and B	Increase in HDL and apo A1 and reduction of BP only in "minerals plus vitamin" group

(continued)

Table 16.7 (continued)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
Regensteiner et al. (2003)	10 premenopausal women with T2D 10 premenopausal controls	Oral L-arginine (9 g/day) Vitamin E (1800 mg/day) + vitamin C (1000 mg/day)	1 week		Flow-mediated brachial artery dilation (FMD) Forearm blood flow	Vitamin supplement was more effective than L-arginine in FMD in diabetic patients No response in controls
Tousoulis et al. (2003)	39 patients with T2D and CVD	Vitamin C (2 g/day) No antioxidant	4 weeks		Forearm vasodilatory response to reactive hyperemia (RH %) PAI-1, vWF, tPA, antithrombin III, protein C and S, factor V, and vII	↑ in RH%, ↓ in factor V, vWF, and tPA in treated group
Beckman et al. (2003)	26 T1D patients 23 T2D patients 45 controls	Vitamin C (1 g/day) + vitamin E (800 IU) Placebo	6 months		Brachial artery endothelium-dependent and endothelium-independent vasodilation	Increased endothelium-dependent vasodilation only in T1D patients
Mullan et al. (2002)	30 T2D	Vitamin C (500 mg/day) Placebo	4 weeks		Brachial systolic and diastolic blood pressure Systemic arterial stiffness (as measured by the central aortic augmentation index) and aortic stiffness (as measured by the time to wave reflection)	Vitamin C decreased systolic and diastolic BP and improved arterial stiffness in diabetics

Darko et al. (2002)	35 T2D patients	Vitamin C (1.5 g/day) Placebo	3 weeks		Oxidative stress (by measuring 8-epi $PGF_{2\alpha}$) BP Endothelial function (assessed by systemic vasodilator response to albuterol and forearm blood flow after Ach)	No effect on BP, oxidative stress, and endothelial function
Heart Protection Study Collaborative Group (2002)	20,536 high-risk people with CAD, other occlusive arterial disease, and diabetes (3982 diabetics)	Vitamin E (600 mg/day) Vitamin C (250 mg/day) β-Carotene (20 mg/day) Placebo	5 years	5 years	All-cause mortality Major coronary events Fatal or nonfatal vascular events Incidence of cancer or other major morbidity	No differences
Nappo et al. (2002)	20 newly diagnosed T2Ds 20 controls	Vitamin E (800 IU) + vitamin C (1 g/day) once before a high-fat meal and once before a high-carbohydrate meal			Measurement of TNF-α, IL-6, ICAM-1, VCAM-1	Vitamin supplement couldn't prevent meal-induced increase in these parameters in diabetics, as it did in controls

ADMA asymmetrical dimethyl arginine, *BS* blood sugar, *CRP* C-reactive protein, *ERP* electron paramagnetic resonance, *FBS* fasting blood sugar, *FBF* forearm blood flow, *GPX* glutathione peroxidase, *HDL* high-density lipoproteins, *ICAM-1* intercellular adhesion molecule-1, *IL-6* interleukin-6, *LDL* low-density lipoprotein, *MDA* malondialdehyde, *NEFAs* nonesterified fatty acids, *ox-LDL* oxidized low-density lipoproteins, *PAI-1* plasminogen activator inhibitor-1, *PBN* alpha-phenyl-tert-butylnitrone, *RBC* red blood cell, *RH%* percent change in reactive hyperemia, *SNP* sodium nitroprusside, *sVCAM* soluble vascular cell adhesion molecule-1, *TNF-α* tumor necrosis factor-α, *TNG%* percent change in nitroglycerin-mediated dilation, *T* tocopherol, *TC* total cholesterol, *TG* triglyceride, *vWF* von Willebrand factor, *WBGD* whole-body glucose disposal

Table 16.8 Selected clinical trials with alpha-lipoic acid in diabetic patients (source Golbidi et al. 2011, with permission)

Reference	Patient and characters	Treatment groups	Treatment duration	Follow-up	Endpoint	Results
Heinisch et al. (2010)	30 T2D patients	ALA (600 mg/day, i.v.) Placebo	21 days		Endothelium-dependent and endothelium- independent vasodilation, assessed by forearm blood flow	ALA improved endothelium-dependent vasodilation
Mittermayer et al. (2010)	30 T2D patients	ALA (600 mg/day, i.v.) Placebo	21 days		Blood level of ADMA (NOS inhibitor)	ALA decreased plasma level of ADMA
Gianturco et al. (2009)	14 T2D patients	ALA (400 mg/day) Placebo	4 weeks		Markers of oxidative stress (assessed by commercially available test, d-ROMs) BAP Lipid profile, CRP	ALA decreased markers of oxidative stress and HDL, had a borderline effect on BAP ($P = 0.06$) and LDL ($P = 0.07$) No significant effect on CRP, TC, and TG
Huang and Gitelman (2008)	40 adolescents with T1D	Controlled release ALA (17 mg/kg/day) Placebo	90 days		8-hydroxy-2′-deoxyguanosine 2-thiobarbituric acid reactive substances Protein carbonyl Total reactive antioxidant potential HbA_{1c} Urine albumin to creatinine ratio	No significant differences in any of the measured parameters
Xiang et al. (2008)	42 subject with impaired glucose tolerance (IGT) test 26 health controls	300 mg ALA before oral glucose tolerance test Placebo			Endothelium-dependent FMD	ALA improved endothelial dysfunction during acute hyperglycemia

Vossler et al. (2007)	114 T2D patients	Tromethamine salt of R-ALA (dexlipotam) (960 mg/day) Dexlipotam (1920 mg/day) Placebo	4 weeks	Percentage change in the FMD of brachial artery	No significant difference in FMD Tendency toward reduction of inflammatory markers and BP
Chang et al. (2007)	50 diabetic patients with end-stage renal disease who undergo hemodialysis (3 times/week)	ALA (600 mg/day) Control group	12 weeks	ADMA TC, hsCRP, oxLDL, albumin, HbA_{1c}	ALA decreased ADMA significantly
Kamenova (2006)	12 T2D patients	ALA (600 mg/day)	4 weeks	– Insulin sensitivity	ALA increased insulin sensitivity in diabetic patients (there was not any significant differences between treated patients and 12 health subjects)
Ziegler et al. (2006)	181 T2D patients	ALA (600 mg/day) ALA (1200 mg/day) ALA (1800 mg/day) Placebo	5 weeks	Evaluation of neuropathic pain based on total symptom score (TSS), neuropathy symptoms and change score, neuropathy impairment score, patients' global assessment	ALA improve neuropathic symptoms (600 mg/day, had the optimum risk to benefit ratio)
Tankova et al. (2004)	46 T1D patients with different forms of autonomic neuropathy	ALA (600 mg/day, i.v.) 10 days followed by ALA (600 mg/day, oral) 50 days Control group	60 days	Scoring different signs and symptoms of autonomic neuropathy Laboratory parameters of oxidative stress	ALA alleviated diabetic autonomic neuropathy Increase in serum antioxidant capacity

(continued)

Table 16.8 (continued)

Reference	Patient and characters	Treatment groups	Treatment duration	Follow-up	Endpoint	Results
Hahm et al. (2004)	38 (out of 61) T2D with symptomatic polyneuropathy	ALA (600 mg/day)	8 weeks		Primary efficacy parameter [total symptom score (TSS) for neuropathic symptoms] Secondary efficacy parameters (clinical neurological assessment, overall rating by the physician and patients at the end of treatment) Laboratory measurements (HbA_{1c}, FBS)	Improvement of polyneuropathy symptoms (decreased TSS score) FBS and HbA_{1c} didn't change
Ametov et al. (2003)	120 T2D patients	ALA (600 mg/day, i.v., 5 days a week for 14 dose) Placebo	Almost 3 weeks		TSS Score of neuropathy signs Score of neuropathy symptoms and change Quantitative sensation tests	ALA significantly improved neuropathic symptoms

ADMA asymmetric dimethylarginine, *BAP* biological antioxidant potential, *CRP* C-reactive protein (CRP), *FBS* fasting blood sugar, *FMD* flow-mediated dilatation, *HDL* high-density lipoproteins, *hsCRP* high-sensitivity CRP, *LDL* low-density lipoprotein, *NOS* nitric oxide synthase, *oxLDL* oxidized LDL, *TC* total cholesterol, *TG* triglyceride

Table 16.9 Selected clinical trials with carnitine in diabetic patients (source Golbidi et al. 2011, with permission)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
Molfino et al. (2010)	16 new diagnosed T2D or IGT	Hypocaloric diet Low hypocaloric diet + carnitine (4 g/day)	10 days		OGTT Fasting plasma insulin Homeostasis model assessment of insulin resistance (HOMA-IR)	OGTT improved in both group Plasma insulin level and HOMA-IR decreased only in carnitine group
Gentile et al. (2009)	40 patients with T2D and erectile dysfunction	One sachet of test formulation (PLC + L-Arg + nicotinic acid) per day (group A) 20 mg vardenafil two times a week (group B) One sachet of test formulation/day plus vardenafil two times a week (group c) Placebo capsules twice a week (group D)	12 weeks		FMD Erectile function (by IIEF5)	2-point increment in IIEF5 in group A, 4 point for group B, 5 point for group C, and no increment of group D
Ruggenenti et al. (2009)	High-risk patients for T2D or metabolic syndrome (−16 patients with GDR ≤7.9 mg/kg/min 16 patients with GDR ≥ 7.9 mg/kg/min)	Oral acetyl L-carnitine (1 g/twice daily)	24 weeks		Glucose disposal rate (assessed by hyperinsulinemic-euglycemic clamps)	Carnitine increased GDR and improved GTT in patient with GDR ≤ 7.9 No effect on those with GDR ≥ 7.9 Decrease systolic BP in both groups Decrease diastolic BP in those with GDR ≥ 7.9
Galvano et al. (2009)	75 patients with T2D	Simvastatin (20 mg/day) Simvastatin (20 mg/day) plus L-carnitine (2 g/day)	4 months		BMI, FPG, HbA_{1C}, TC, LDL, HDL, TG, apolipoprotein A1, Apo B, lipoprotein (a), and apoprotein (a)	Decrease in TG, FBG, Apo B, Lp (a), apo (a) and increase in HDL in combined group

(continued)

Table 16.9 (continued)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
Malaguarnera et al. (2009a)	80 T2D patients	Simvastatin (20 mg/day) Simvastatin (20 mg/day) plus L-carnitine (2 g/day)	12 weeks		BMI, FPG, TC, LDL, LDL subclasses and LDL size, HDL, TG, apolipoprotein A1, apolipoprotein B-100, HbA_{1c}	Small sized LDL particles decreased only in combined group
Malaguarnera et al. (2009b)	81 T2D patients	L-Carnitine (2 g/day) Placebo	3 months		BMI, HbA_{1c}, FBG, TC, LDL, HDL, TG, apolipoprotein A1, apolipoprotein B-100, oxLDL, TBARS, conjugated dienes	L-Carnitine reduced oxLDL, LDL, TG, apolipoprotein A1, apolipoprotein B-100, TBARS, and conjugated diene
González-Ortiz et al. (2008)	12 T2D patients	L-carnitine (3 g/day) Placebo	4 weeks		Insulin sensitivity (by euglycemic-hyperinsulinemic clamp) Lipid profile	No differences between two groups
Morano et al. (2007)	32 T2D with erectile dysfunction	PLC (2 g/day) PLC (2 g/day) + sildenafil (50 mg/day twice weekly) Sildenafil (50 mg/day) Placebo	12 weeks	4 weeks posttreatment	Monocyte oxidative activity [stimulation index (SI)] ICAM-1 P-selectin Advance glycation end product Doppler sonography IIEF-5	SI was reduced in PLC group and combined treatment Reduction of ICAM, P-selectin, end diastolic velocity in combined treatment IIEF-5 improved in sildenafil and combined group IIEF-5 improvement was maintained 4 weeks posttreatment in combined group

Santo et al. (2006)	74 T2D patients with peripheral arterial disease	PLC (2 g/day) Placebo	12 months	Ankle/brachial index (ABI) Pain-free walking distance Malondialdehyde, 4-hydroxynonenal, oxidation time of LDL, nitrite/nitrate ratio	ABI improved progressively, pain-free walking distance increased and improvement in all oxidative parameters in treated group
Solfrizzi et al. (2006)	52 T2D with Lp(a) ≥ 20 mg/dL	Simvastatin (20 mg/day) Simvastatin (20 mg/day) + L-carnitine (2 g/day)	2 months	TG, TC, LDL, HDL, apoB, Lp(a)	No difference in LDL, non-LDL cholesterol, and apoB Lp(a) increased in simvastatin group but decreased in combined group
Uzun et al. (2005)	51 T1D and 21 healthy controls	L-carnitine (2 g/day)	2 months	NCV SSR	Patient with pathologic NCV and normal neurologic exam showed 44% and 50% improvement in NCV and SSR parameters, respectively Patient with pathologic NCV and neurologic exam just had improvement in SSR
Rahbar et al. (2005)	35 T2D	L-carnitine (1 g, tid) Placebo	12 weeks	FBG and lipid profile	FBG decreased in treated group TG, Apo A1, Apo B-100 increased in treated group No change in LDL, HDL, LP(a), TC, HbA_{1c}

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Table 16.9 (continued)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
Sima et al. (2005)	1257 T2D patients with neuropathy	Acetyl L-carnitine (ALC) (500 mg/tid) ALC (1 g/tid) Placebo	52 weeks		Morphometric analyses of sural nerves Electrophysiological parameters Vibration perception Clinical symptom score and visual analogue scale score	Improvement in sural nerve fiber numbers and regeneration and also vibration perception No improvement in NCV and amplitudes Improvement in pain as a bothersome symptom (especially with 1 g dose)
Gentile et al. (2004)	40 diabetic (T1&2D) with erectile dysfunction and refractory to sildenafil monotherapy	Sildenafil (50 mg twice weekly) Sildenafil (50 mg twice weekly) + PLC (2 g/day)	24 weeks		– IIEF	Combined therapy was more effective than sildenafil monotherapy
Ragozzino et al. (2004)	24 T2D with PAD and 20 T2D without PAD	PC (600 mg/twice daily, i.v.) after 7 days of hypocaloric diet Placebo	10 days		PAD-related symptoms Glycemic control	Increase in max. walking distance and initial claudication distance in treated group Decrease in dosage of oral antihyperglycemic drugs in treated group
Derosa et al. (2003)	94 newly diagnosed T2D with hypercholesterolemia	L-Carnitine (1 g/bid) Placebo	6 months		BMI, FBG, postprandial plasma glucose, HbA_{1c}, FPI, TC, LDL, HDL, TG, apo A-I, apo B, Lp(a)	Lp(a) decreased in treated group No change in other parameters

De Grandis and Minardi (2002)	333 T2D patients with diabetic neuropathy	Acetyl-L-carnitine (LAC) (1 g/day, I.M. followed by 2 g/day, p.o.)	1 year	NCV Amplitude in the sensory (ulnar, sural, and median) and motor (median, ulnar, and peroneal) nerves Effect on pain (by VAS)	Treated group showed improvement in NCV (at 12 months) and amplitude and reduced VAS

BP blood pressure, *BMI* body mass index, *FPI* fasting plasma insulin, *FPG* fasting plasma glucose, *FMD* flow-mediated dilatation, *GDR* glucose disposal rate, *HDL* high-density lipoproteins, *IGT* impaired glucose tolerance, *ICAM-1* intercellular adhesion molecule-1, *IIEF-5* international index of erectile function questionnaire, *LDL* low-density lipoproteins, NCV nerve conduction velocity, OGTT oral glucose tolerance test, *oxLDL* oxidized LDL, *PAD* peripheral arterial disease, *PLC* propionyl-L-carnitine, *SSR* sympathetic skin responses, *TBARS* thiobarbituric acid reactive substances, *TC* total cholesterol, *TG* triglyceride, *VAS* visual analogue scale

Table 16.10 selected clinical trials with CoQ10 in diabetic patients (source Golbidi et al. 2011, with permission)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
Palacka et al. (2010)	59 T2D patients	Polarized light (PL) therapy (n = 19) 10 min, twice daily Antioxidants [CoQ10 60 mg + ALA 100 mg + vitamin E 200 mg] bid (QALA) PL + QALA	3 month		CRP LDH MDA Serum antioxidant level (CoQ10, α- and τ-tocopherol, β-carotene) Echocardiographic parameters of left ventricular function	Combined therapy had better results in increasing the antioxidant level, decreasing MDA, and improving left ventricular function
Hamilton et al. (2009)	23 statin-treated T2D	CoQ10 (200 mg/day) Placebo	12 weeks		Improvement of endothelial function (as measured by brachial artery FMD) Nitrate-mediated dilation Plasma F2-isoprostane and urinary 20-hydroxyeicosatetraenoic acid (HETE)	CoQ10 improved endothelial function but did not alter plasma F2-isoprostane or 20-HETE
Chew et al. (2008)	74 patients with T2D	Fenofibrate 160 mg/day CoQ 200 mg/day Fenofibrate + CoQ Placebo	6 months		Ambulatory blood pressure Heart rate Diastolic function (measured by echo)	Neither fenofibrate nor CoQ (alone or in combination) improved diastolic function CoQ and fenofibrate independently lowered 24-h blood pressure
Lim et al. (2008)	80 T2D (Asian)	CoQ 200 mg/day Placebo	12 weeks		Soluble intercellular adhesion molecule Plasma α-tocopherol Ubiquinol-10 Total CoQ10 Cutaneous microcirculatory function (by laser Doppler) in response to Ach and SNP	CoQ10 was ineffective in improving microcirculatory endothelial function

Hodgson et al. (2002), Playford et al. (2003)	80 dyslipidemic T2D patients	Fenofibrate (200 mg/day) CoQ (200 mg/day) Fenofibrate (200 mg/day) + CoQ (200 mg/day) Placebo	12 weeks	Endothelium-dependent and endothelium-independent forearm microcirculatory function plus lipid profile and glycemic controls	CoQ and fenofibrate alone did not change vascular reactivity Combined treatment improved endothelium-dependent and endothelium-independent vasodilation which was correlated with HbA_{1c} CoQ did not change plasma isoprostane, but lowered systolic BP
Watts et al. (2002)	40 dyslipidemic T2D patients 18 nondiabetic controls	CoQ 200 (mg/day) Placebo	12 weeks	Endothelium-dependent and endothelium-independent function of brachial artery Plasma F2-isoprostane Plasma antioxidant status Lipid profile and glycemic control	CoQ improved endothelial function in conduit arteries CoQ did not alter plasma F2-isoprostane, plasma antioxidant status, lipid profile, glycemic control, and BP

Ach acetylcholine, *CRP* C-reactive protein, *FMD* flow-mediated dilatation, *LDH* lactate dehydrogenase, *MDA* malondialdehyde, *SNP* sodium nitroprusside

Table 16.11 Selected clinical trials with ruboxistaurin in diabetic patients (source Golbidi et al. 2011, with permission)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
Beckman et al. (2010)	13 T2D patients without CVD 15 healthy controls	Ruboxistaurin (32 mg/day, p.o.) Placebo	14 days		Endothelium-dependent and endothelium-independent vasodilation of forearm resistance vessels Plasma markers of inflammation, fibrinolysis, endothelial damage, and oxidative stress	No effect on either of the evaluated parameters
Mehta et al. (2009)	52 T2D patients	Ruboxistaurin (32 mg/day) Placebo	6 weeks		FMD of the brachial artery assessed by ultrasound Urinary levels of isoprostane, 8,12-iso-iPF$_{2\alpha}$-VI	Ruboxistaurin tended to improve FMD no change in urinary isoprostane
Brooks et al. (2008)	20 T1&2D patients with diabetic peripheral neuropathy	Ruboxistaurin (32 mg/day) Placebo	1 year		SkBF (by laser Doppler velocimetry combined with iontophoresis of Ach and SNP)	No significant differences were observed
Tuttle et al. (2007)	1157 diabetic patients (T1&2D) with eye disease	Ruboxistaurin (8, 16, and 32 mg/day) Placebo	30–36 months		Kidney outcomes (including doubling of serum creatinine, development of advanced chronic kidney disease, and death)	Results were similar between two groups
Casellini et al. (2007)	40 T1&2D with DPN	Ruboxistaurin (32 mg/day) Placebo	6 months		Endothelial-dependent and C fiber-mediated SkBF, sensory symptoms, neurological deficits, nerve fiber morphometry, autonomic function testing, NCV, quality of life	Ruboxistaurin-enhanced SkBF, reduced sensory symptom

PKC-DMES Study Group (2007)	686 T1&2D with DME	Ruboxistaurin (4, 16, or 32 mg/day) Placebo	30 months	Progression to sight-threatening DME Application of focal/grid photocoagulation for DME	No delay in progression to primary outcomes
Gilbert et al. (2007)	107 T2D with diabetic nephropathy	Ruboxitaurin (32 mg/day)	52 weeks	Change in urinary level of TGF-β	Prevented increase in urinary TGF-β
PKC-DMES Study Group (2006)	685 T1&2D patients with diabetic retinopathy	Ruboxitaurin (32 mg/day)	36 months	Evaluation of sustained moderate visual loss in patients with moderately severe to very severe nonproliferative diabetic retinopathy	Reduced vision loss, need for laser therapy, and macular edema progression
Aiello et al. (2006)	29 T1&2D patients	Ruboxitaurin (16 mg/day) Ruboxitaurin (32 mg/day) Placebo	28 days	Mean retinal circulation time (RCT) Retinal blood flow (RBF)	Ameliorate diabetes-induced retinal hemodynamic abnormalities
Tuttle et al. (2005)	123 T2D patients	Ruboxitaurin (32 mg/day) with persistent albuminuria Placebo	1 year	Change in ACR Calculation of eGFR	Reduced albuminuria and maintained eGFR
Vinik et al. (2005)	205 T1&2D patients with DPN	Ruboxitaurin (32 mg/day) Ruboxitaurin (64 mg/day) Placebo	1 year	Change in VDT NTSS-6	No significant change in VDT and NTSS-6
Strøm et al. (2005)	41 T1&2D with DME	Ruboxitaurin (4 mg/day) Ruboxitaurin (16 mg/day) Ruboxitaurin (32 mg/day) Placebo	18 months	Retinal vascular leakage (vitreous fluorometry)	Significant reduction in retinal vascular leakage in those with baseline permeability ≥5.8 nm/s

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Table 16.11 (continued)

Reference	Patient and characters	Treatments groups	Treatment duration	Follow-up	Endpoint	Results
PKC-DRS Study Group (2005)	252 T1&2D with moderately severe to very severe nonproliferative diabetic retinopathy	Ruboxitaurin (8 mg/day) Ruboxitaurin (16 mg/day) Ruboxitaurin (32 mg/day) Placebo	36–46 months		Progression of diabetic retinopathy Moderate visual loss (MVL) Sustained MVL	No significant effect on progression of diabetic retinopathy 32 mg/day was associated with delayed occurrence of MVL

Ach acetylcholine, *ACR* albumin to creatinine ratio, *CVD* cardiovascular disease, *DME* diabetic macular edema, *DPN* diabetic peripheral neuropathy, *eGFR* estimated glomerular filtration ratio, *FMD* flow-mediated dilation, *SkBF* skin microvascular blood flow, *SNP* sodium nitroprusside (SNP), *TGF-β* transforming growth factor β, *T1&2D* type 1 and 2 diabetes, *VDT* vibration detection threshold

16.4 Conclusions

Although though there is no doubt about the critical role of oxidative and inflammatory stress in the pathogenesis of diabetes, the lack of clear-cut clinical benefits with antioxidant use is a major disappointment. This failure emphasizes the fact that we need to better appreciate the translation of molecular findings from experimental studies to clinical practice by addressing fundamental questions of some of the reasons for this disparity. To our understanding there are several possibilities that should be taken into account. First, oxidative radicals are not all harmful and not every antioxidant agent is beneficial. For instance, reactive oxygen species are critical for cellular process such as immunity, growth and differentiation, or intracellular signaling. Antioxidants can inadvertently affect these phenomena. In addition, some antioxidants can act as prooxidant agents after neutralizing oxidative radicals, for example, vitamin E is transformed to tocopheroxyl radicals after scavenging free radicals. Several other antioxidants are needed for de novo regeneration of vitamin E. Therefore, targeting specific reactions rather than inhibiting a whole chain reaction is an important consideration for achieving better clinical outcomes. Second, variations in genetic backgrounds are an important confounding factor in clinical trials, for example, as is the case for vitamin C and the haptoglobin genotype where genetic variations appear to explain some of the contradictory results in clinical trials.

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Chapter 17
Prevention of Type 2 Diabetes by Polyphenols of Fruits

H.P. Vasantha Rupasinghe, Nileeka Balasuriya, and Yanwen Wang

Abstract Type 2 diabetes is a metabolic disorder, which leads to a series of complications in the human body including damage to the heart, blood vessels, brain and neurons, kidneys, eyes, toes, skin, and mouth. Dietary intervention with phenolic compounds has been studied extensively for the prevention of type 2 diabetes. Phenolic compounds are abundant in fruits. Phenolic extracts and isolated polyphenols from fruits have been demonstrated to have antidiabetic effects through a large number of studies with different settings, including cell-based experiments, epidemiological analyses and studies, preclinical animal studies, and human clinical trials. The mechanisms of action of antidiabetic effects of phenolic compounds include the improvement in impaired insulin sensitivity, inhibition of carbohydrate-hydrolyzing enzymes, and upregulation of glucose transport, antioxidant, and anti-inflammation. This chapter provides recent evidence on the physiological functions of fruit polyphenols in reducing the risk of type 2 diabetes in different experimental settings and model systems.

Keywords Diabetes • Insulin resistance • Hyperglycemia • Fruits • Phenolic compounds

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17.1 Introduction

The incidence of diabetes increased rapidly in the past decade. According to the report issued by the World Health Organization in 2016, 422 million adults in the world live with diabetes, mainly in low- and middle-income countries (World Health Organization 2016). Diabetes has become a world epidemic and together with complications affects dramatically the quality of life and socioeconomic and health-care systems. Prevention of diabetes, especially type 2 diabetes (T2D), has become critically important, at least in part through change in dietary habits and lifestyle behaviours.

Pathogenesis of T2D is characterized by insulin resistance in the peripheral tissues such as muscle and fat. With the development of insulin resistance, the body becomes less responsive to insulin, and thus, the glucose intake by skeletal muscle and adipose tissue decreases. However, intestinal glucose absorption and liver glucose production are not suppressed, resulting in an elevation of the circulating glucose levels, known as hyperglycemia (Stumvoll et al. 2005). Although insulin resistance can be compensated by increasing insulin production and secretion in the pancreatic β-cells, the ability of these cells to secrete insulin is impaired with the progression of insulin resistance if it is not corrected, and eventually, results in β-cell exhaustion and dysfunction. Several reviews (Lin and Sun 2010; Yabe et al. 2015; King and Bowe 2016) have summarized the pathogenesis of T2D in association with the tissues and organs involved in glucose metabolism and homeostasis.

Malfunction of adipose tissue under conditions of obesity plays a vital role in the development of insulin resistance. Adipose tissue is traditionally considered as a place for fat and energy storage. Emerging evidence has shown that adipose tissue produces a number of cytokines named adipokines or adipocytokines, and many of which are pro-inflammatory and can create a chronic state of inflammation and antagonize the insulin signaling (Qureshi and Abrams 2007). In obesity, adipocytes continuously release fatty acids into the bloodstream, which can cause accumulation of fat in other tissues and organs. This phenomenon is called lipotoxicity, which is an important cause of insulin resistance. Skeletal muscle is the principle tissue involved in glucose homeostasis and responsible for more than 70% of insulin-stimulated glucose uptake (Lin and Sun 2010). Insulin stimulates transportation of glucose into cells by activating insulin receptor substrate-1 (IRS-1)-dependent phosphatidylinositol 3-kinase (P13K), which activates atypical protein kinase C (aPKC) and protein kinase B (Akt) (Fig. 17.1). Activated Akt interacts with glucose transporter (GLUT) isoforms, especially GLUT4, which then translocates from the cytoplasm to the cell membrane and transport glucose across the membrane into the cell (Whiteman et al. 2010). Insulin resistance results from the impairment of tyrosine phosphorylation of IRS-1 and abnormalities of aPKC/Akt activation in skeletal muscle and thus GLUT translocation (Farese et al. 2005).

Hepatic glucose production is another important contributor to glucose homeostasis. Insulin increases glucose uptake and glycogen synthesis in liver cells and suppresses phosphoenolpyruvate carboxy kinase (PEPCK) and glucose-6-phosphatase (G6Pase) gene expressions by activating PI3K. When insulin resistance occurs, the

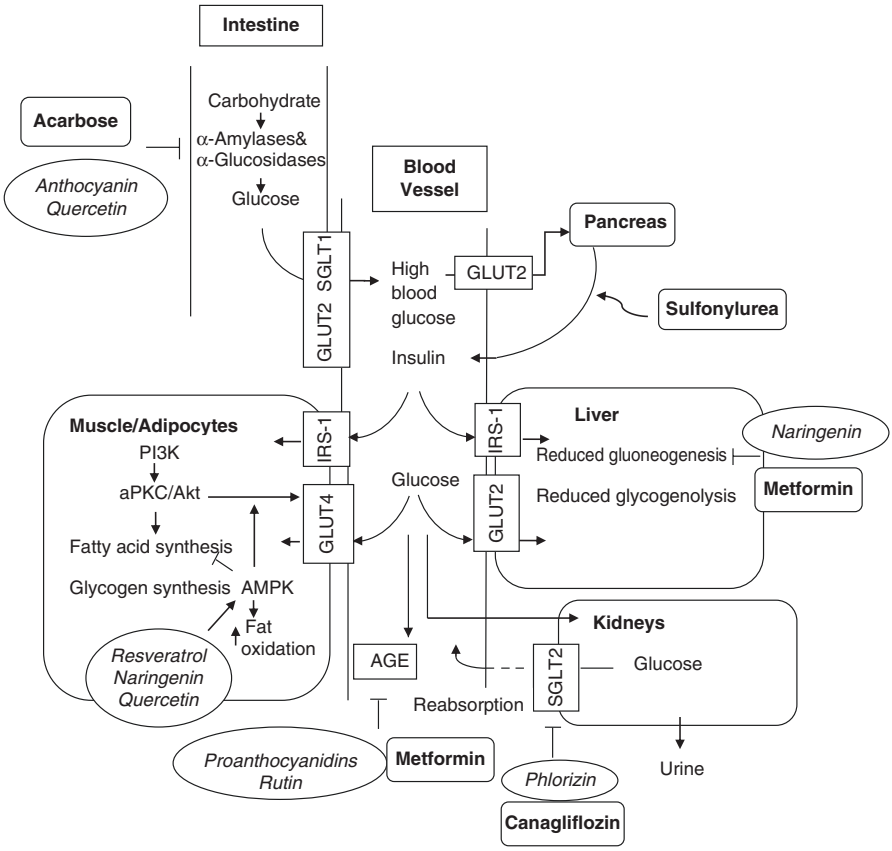

Fig. 17.1 Insulin-mediated glucose metabolism in different organs and tissues. *SGLT1/2* sodium-dependent glucose transporters 1 and 2, *IRS-1* insulin receptor substrate, *PI3K* phosphatidylinositol 3-kinase, *Akt* protein kinase b, *aPKC* atypical protein kinase c, *AMPK* AMP-activated protein kinase, *AGE* advanced glycation end-products

expression and activity of these two gluconeogenic enzymes are upregulated, resulting in an increase of hepatic of glucose production (Whiteman et al. 2010). Insufficiency of insulin secretion and/or insulin resistance results in over- or uncontrolled production of hepatic glucose, which together with decreased peripheral glucose uptake and disposal contributes to hyperglycemia.

17.2 Drugs for the Treatment of Type 2 Diabetes

Drugs used to treat T2D have a variety of mechanisms of action (Fig. 17.1). Increasing insulin secretion, decreasing glucose absorption in the intestine and renal filtrate, increasing glucose uptake by the peripheral tissues, and decreasing

hepatic glucose production are the main targets of currently available antidiabetic drugs (Bedekar et al. 2010; Ahuja and Chou 2016). Metformin (*N,N*-dimethylimidodicarbonimidic diamide) is the most widely studied and used drug for treating T2D (Ahmed and Crandall 2010), which inhibits hepatic gluconeogenesis and increases peripheral glucose uptake. Thiazolidinediones reduce hyperglycemia through several different mechanisms, including activating peroxisome proliferator-activated receptor-gamma (PPAR-**γ**) in the adipose tissue and thus altering the adipose metabolism, reducing the circulating levels of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), and increasing adiponectin levels (Stumvoll et al. 2005). Acarbose, miglitol, and voglibose are examples of digestive enzyme inhibitors. These drugs typically inhibit α-glucosidase and/or α-amylase and thus limit glucose uptake by reducing or delaying carbohydrate digestion (Bedekar et al. 2010). Sulfonylurea derivatives (e.g., glipizide, gliclazide, glibenclamide, glibornuride) improve insulin secretion by closing potassium channels in pancreatic β-cells.

Recently approved antidiabetic drugs include incretin-based therapies such as peptides glucagon-like peptide-1 (GLP-1) receptor agonists (Saxenda™, Liraglutide™) and dipeptidyl peptidase-4 (DPP4) inhibitors or gliptins, for example, Alogliptin™ (Egger et al. 2016). GLP-1 is a potent antihyperglycemic intestinal hormone, which stimulates β-cells of the pancreas to release insulin in response to rising glucose. However, GLP-1 has a half-life of less than two minutes, due to rapid degradation by DPP4. Clinical data on the safety of incretin-based therapies is limited; however, it seems that incretin-based therapies are not increasing the risk of bone fracture. Canagliflozin (Invokana™), dapagliflozin (Forxiga™), empagliflozin (Jardiance™), inhibitors of sodium-glucose co-transporter 2 (SGLT2) of kidney, are also a relatively new class of drugs for T2D (Egger et al. 2016). The main mechanisms of action of these drugs include glucose reabsorption from the kidney and increase in renal glucose excretion. However, elevated cardiovascular disease (CVDs) risk of patients treated with SGLT2 inhibitors has been reported (Egger et al. 2016).

Although offering the beneficial effects of controlling diabetes, most drugs are associated with side effects such as increases in blood pressure, blood lipids, and body weight (Mehanna 2013). As such, many diabetic patients are treated in combination with antihypertensive and/or lipid-lowering drugs (Stumvoll et al. 2005). Natural health products are gaining popularity due to having fewer side effects or no side effects at all, in addition to the cost of development and use compared to drugs (Mehanna 2013). Plant secondary metabolites, including phenolic acids, flavonoids, carotenoids, terpenes, and alkaloids are potential antidiabetic agents.

17.3 Fruit Polyphenols

Polyphenols are a large group of secondary metabolites that protect fruits against environmental stress conditions (Rupasinghe 2008; Parmar and Rupasinghe 2015). The diverse group of phenolic compounds includes simple phenols,

phenylpropanoids, benzoic acid derivatives, cinnamic acid derivatives, flavonoids, stilbenes, and lignans. Numerous health benefits of phenolic compounds have been reviewed (Yao et al. 2004; Thilakarathna and Rupasinghe 2012; Rupasinghe et al. 2013; Bhullar and Rupasinghe 2013; George et al. 2017), with the primary focus on their antioxidant properties. In North American diets, the intake of polyphenols is mainly from apples (*Malus domestica* Borkh.), which along with strawberries (*Fragaria* × *ananassa* Duchesne) have high antioxidant contents and activities (Wolfe et al. 2008). Most fruits are rich in flavonoids, and berries are well known for their high contents of flavonoids (Häkkinena et al. 1999). Catechins and proanthocyanidins are prominent in blackberries (*Rubus fruticosus* L.), strawberries, and blueberries (*Vaccinium corymbosum* L.). The anthocyanin content is higher in blueberries than other types of berries (Huang et al. 2012). Anthocyanins offer a number of health benefits such as antioxidant, anticancer, and antidiabetes (Sancho and Pastore 2012). In apple, cyanidin and quercetin glycosides are prominent flavonoids, which present predominantly in the peel (Huber and Rupasinghe 2009). Citrus flavonoids have been categorized into three main subgroups: flavone glycosides, polymethoxyflavones, and flavanones. Oranges (*Citrus sinensis* L. Osbeck), tangerines (*Citrus tangerine* Tanaka), and lemons (*Citrus limonum* L.) are rich in rutinosides (e.g., hesperidin, narirutin, eriocitrin, and diosmin) and polymethoxyflavones (e.g., tangeretin, nobiletin, and hepatomethoxyflavones) (Hwang et al. 2009). Flavones occur in lemons and are not present in other citrus fruits (Hwang et al. 2009). A study comparing three tropical fruit extracts for their flavonoid contents has shown that guava (*Psidium guajava* L.) and banana (*Musa paradisiaca* L.) have higher concentrations of flavonoids than pineapple (*Ananas comosus* L. Merr) (Alothman et al. 2009). Trans-resveratrol, from the stilbene group of compounds, is abundant in grapes (*Vitis vinifera* L.) (Ratnasooriya and Rupasinghe 2012) and is a cardioprotective and chemopreventive phenolic compound.

Since oxidative stress plays an important role in the pathogenesis of chronic diseases, the efficacy of plant polyphenols in reducing oxidative stress and stress-mediated disease conditions have been investigated extensively. Most studies support the fact that long-term intake of polyphenol-rich foods, such as fruits and vegetables, is beneficial in preventing or delaying the onset of chronic diseases. Accordingly, increasing efforts have been put on the development of functional foods and nutraceuticals that incorporate specific plant polyphenols with scientifically proven health benefits. Regardless of the method or route of intake, one major issue associated with these compounds is the poor bioavailability (Thilakarathna and Rupasinghe 2013).

17.3.1 Bioavailability of Fruit Polyphenols

Polyphenols are quickly metabolized once ingested. Among the structural characteristics of the polyphenols, the presence of sugar moieties and the degree of polymerization are the main factors that determine the absorption, metabolism, and

circulating concentrations of polyphenols (Manach et al. 2004). The presence of a sugar moiety in flavonols, such as in quercetin, favors the absorption of flavonols. The absorption rate of quercetin glycoside is ten times higher than aglycone quercetin. The most readily absorbable flavonoids is the monomeric form. Since the molecule size increases with polymerization, the absorption rate of polymer forms of flavonoids is decreased. Isoflavonoids are the best-absorbed flavonoids among all (Manach et al. 2004). Anthocyanins have the least bioavailability compared to other flavonoids (Rupasinghe et al. 2013; Thilakarathna and Rupasinghe 2013).

Monomeric flavonoids can undergo metabolic conversions in the enterocytes post absorption. Glycosylated phenolics are deglycosylated by lactase phlorizin hydrolase (LPH) and β-glucosidase in the lumen and in the enterocytes respectively following absorption. Quercetin glycosides, including quercetin glucoside, quercetin galactoside, quercetin rutinoside, and quercetin rhamnoside, are deglycosylated into quercetin molecules. After reaching the liver, these molecules undergo a series of metabolic conversions, including methylation, glucuronidation, and sulfation in the hepatocytes (Rupasinghe et al. 2010; Thilakarathna and Rupasinghe 2013), resulting in the production of isorhamnetin-3-*O*-glucuronic acid, quercetin-3-*O*-glucuronic acid, and quercetin-3′-sulfate. Human neutrophils can deconjugate these metabolites, especially glucuronide conjugation through the action of β-glucuronidase to regenerate aglycones of flavonoids (Perez-Vizcaino et al. 2012). The activity of β-glucuronidase is enhanced under some oxidative stress-related conditions such as inflammation (Shimoi and Nakayama 2005). The biologically available phenolic metabolites not only act as antioxidants but also as mediators of signal transduction pathways and regulators of antioxidant enzyme activity. These distinct characteristics of polyphenols have gained attention recently in the prevention and treatment of chronic disease conditions including T2D.

17.3.2 In Vitro Antidiabetic Effects of Fruit Polyphenols

Fruit extracts and isolated polyphenols have been demonstrated to have antidiabetic effects through a large number of in vitro studies. The mechanisms of action of antidiabetic effects of phenolic compounds include inhibition of carbohydrate hydrolysis, inhibition of protein glycation, and other mechanisms.

17.3.2.1 Inhibition of Carbohydrate Hydrolysis

Inhibition of carbohydrate-hydrolyzing enzymes such as α-glycosidase and α-amylases in the gut is one of the strategies in the treatment of T2D (Fig. 17.1). Although many enzyme inhibitors are derived from microorganisms (Bedekar et al. 2010), berry phenolics have widely been studied in vitro for their inhibitory activities on α-glucosidase and α-amylase (Table 17.1). Highbush blueberries and wine produced from blueberries inhibit α-amylase and α-glucosidase enzymes (Johnson

Table 17.1 Antidiabetic mechanisms of fruit extracts in vitro

In vitro bioactivity	Mechanism of action	Fruit	Bioactives	References
Carbohydrate hydrolyzation	Inhibition of α-glucosidase	Blueberry	Anthocyanins	Johnson et al. (2011)
		Cranberry	Quercetin, coumaric acid derivatives	Pinto Mda et al. (2010)
	Inhibition of α-amylase	Grape seed	–	Liu et al. (2011)
		Strawberry	Tannins	Cheplick et al. (2010)
		Raspberry	Anthocyanins	Grussu et al. (2011)
		Rowanberry	Proanthocyanidins	Grussu et al. (2011)
		Cranberry	Quercetin, coumaric acid derivatives	Pinto Mda et al. (2010)
Protein glycation	Inhibition of AGE production	Grape seed	–	Liu et al. (2011)
	Inhibition of AGE-induced cell proliferation	Blueberry, blackberry, strawberry, raspberry, tomato paste	Procyanidins Rutin	Wang et al. (2011) Kiho et al. (2004)
		Citrus fruits	–	Ramful et al. (2010)
		Grape seed	–	Cai et al. (2011)

et al. 2011). Blueberry and blackcurrant (*Ribes nigrum* L.) extracts rich in anthocyanins are better inhibitors of α-glucosidase than of α-amylase. Proanthocyanidins and tannins inhibit α-amylase (Grussu et al. 2011). Similar effects were observed for strawberries, raspberries (*Rubus fruticosus* L.), and rowanberries (*Sorbus aucuparia* L.) (Grussu et al. 2011; Cheplick et al. 2010). In contrast, Grussu et al. (2011) reported that ellagitannins although present at a high concentration in yellow raspberries did not inhibit α-amylase activity. Cranberry (*Vaccinium macrocarpon* L.) extracts rich in quercetin and *p*-coumaric acid derivatives inhibit both α-glucosidase and α-amylase, with greater inhibition being seen on α-glucosidase (Pinto Mda et al. 2010). Polyphenol-rich wild berry stem infusions also inhibit α-glucosidase and α-amylase activity in vitro (Parmar and Rupasinghe 2015).

Polyphenols enhance the effects of the antidiabetic drug acarbose, a carbohydrate-hydrolyzing enzyme inhibitor. For example, proanthocyanidin-rich fractions of rowanberries reduce the dose of acarbose needed for blood glucose control (Grussu et al. 2011). This beneficial effect of polyphenols applies to other berry extracts as well. Apart from berries, citrus fruit polyphenols also inhibit carbohydrate-hydrolyzing enzymes (Menichini et al. 2011; Liu et al. 2011). Grape seed extracts inhibit rat intestinal α-glucosidase and porcine α-amylase, and the inhibition of α-amylase by grape seed extracts is almost four times greater than acarbose (Liu et al. 2011). Overall, fruit extracts rich in flavonoids are able to improve hyperglycemia by reducing or delaying glucose absorption via inhibiting enzymes responsible for carbohydrate hydrolysis or digestion in the gut.

17.3.2.2 Inhibition of Protein Glycation

A series of non-enzymatic reactions between sugars and free amino groups of proteins, lipids, and nucleic acids produce advanced glycation end-products (AGEs) in humans. Many complications associated with diabetes, atherosclerosis, and aging result from the accumulation of AGEs (Peng et al. 2011). Antidiabetic drugs, such as metformin, repress AGE formation (Beisswenger and Rugeiro-Lopez 2003). In addition to drugs, polyphenols are being increasingly investigated for their ability to prevent AGE formation (Table 17.1). A number of studies examined the effect of fruit polyphenols on AGE production. Studies using in vitro models demonstrate that blueberry, blackberry, strawberry, raspberry extracts, and tomato extracts inhibit the formation of AGE (Kiho et al. 2004; Wang et al. 2011). Citrus fruit (tangor Elendale and tangelo Minneola) extracts reduce protein carbonyls in albumin glycation in a human liposarcoma (SW872) cell line (Ramful et al. 2010). A grape seed extract inhibits AGE-induced cell proliferation in a vascular smooth muscle cell line (Cai et al. 2011). Polyphenols have been identified as key bioactive components in these fruit extracts responsible for the inhibitory effects of protein glycation. In berry and grape seed extracts, proanthocyanidins are predominant bioactives, but in tomato extract, quercetin-3-*O*-rutinosides (rutin) are dominated (Kiho et al. 2004; Cai et al. 2011). In another study, it was observed that rutin and its five metabolites reduced glucose oxidation and collagen-1 glycation in a dose-dependent manner (Cervantes-Laurean et al. 2006).

Most flavonoids possess inhibitory properties on AGE production. For instance, quercetin, isoquercitrin, hyperoside (quercetin-3-*O*-galactoside), and cacticin (isorhamnetin-3-*O*-galactoside) decrease AGE formation (Kim et al. 2011). The structure-activity relationship between flavonoids and AGE production indicates that flavones show stronger effects than flavonols, flavanones, and isoflavones. Hydroxyl groups at C-3′ were more important than C-3 hydroxyl groups, and a positive relationship was observed between the number of hydroxyl groups at C-4′, C-5, and C-7 positions and AGE inhibition (Peng et al. 2011; Matsuda et al. 2003). Ferulic acid produces more than 90% inhibition of AGE formation and regulates the formation of early Maillard reaction products (Silván et al. 2011). The in vitro evidence indicates that fruit flavonoids prevent diabetes partially through inhibiting protein glycation, an important mechanism in the development of diabetes and its complications.

17.3.2.3 Other Mechanisms

Blueberry extracts and juices have been investigated for their effects on diabetes using cell models (Table 17.2). Fermented blueberry extracts increased glucose uptake by 48% in C2C12 skeletal myotubes in the presence and absence of insulin, whereas non-fermented blueberry juice did not show any effect (Vuong et al. 2009). The glucose transporters in C2C12 skeletal myotubes are insulin regulated. Blueberry root, stem, leaf, and fruit extracts all affect insulin sensitivity, whereas

Table 17.2 Antidiabetic properties of fruit extracts and fruit polyphenols in different cell model systems

Fruit extracts/ phenolics	Biological property	Mechanism of action	Cell model	References
Blueberry (fermented juice)	Hypoglycemic effect	Increase glucose uptake by cells	C2C12 myotubes	Vuong et al. (2009)
	β-Cell proliferation	Reduce cytotoxicity	β–TC-tet cells	Martineau et al. (2006)
Grape powder extract	Reduce inflammation	Down-regulation of IL-6, IL-1β, IL-8, MCP-1, COX-1, TLR-2	Adipocytes	Chuang et al. (2011)
Chinese bayberry extract	β-Cell proliferation	Protect against necrosis and apoptosis	Pancreatic β-cells	Zhang et al. (2011)
Acerola fruits	Hypoglycemic effect	Inhibition of α-glucosidase and glucose transporters	Caco-2 cells	Hanamura et al. (2006)
Quercetin	Anti-adipogenic activity	Activation of AMPK pathway	3T3-L1 preadipocytes	Ahn et al. (2008)
Naringenin	Hypoglycemic effect	Activation of AMPK pathway Inhibition of glucose uptake (intestine and kidney)	L6 rat myotubes Intestinal brush border membrane vesicles	Zygmunt et al. (2010) Li et al. (2006)
Phlorizin	Inhibition of glucose transportation	Inhibition of SGLT2	COS-7 cells	Pajor et al. (2008)

only the root, stem, and leaf extracts improve insulin sensitivity in C2C12 myotubes (Martineau et al. 2006). The bioactive compounds responsible for these antidiabetic effects were not identified, but it is likely attributed to the high content of anthocyanins. Indeed, animal studies and clinical trials demonstrated that supplementation with anthocyanins improves insulin resistance, protect β-cells, increase secretion of insulin, and reduce carbohydrate digestion in the gut (Sancho and Pastore 2012).

The activation of inflammation-mediated pathways and gene expressions is a causative factor in insulin resistance (Evans et al. 2002). Grape powder extracts attenuate inflammation and insulin resistance in human adipocytes. When human adipocytes were co-cultured with TNF-α and grape powder extracts, the expression of interleukin (IL)-1β, IL-8, monocyte chemoattractant protein (MCP-1), cyclooxygenase (COX)-1, and toll-like receptor (TLR)-2 was reduced in a dose-dependent manner compared to cells cultured with TNF-α alone (Chuang et al. 2011). Furthermore, grape powder extracts attenuate the expression of tyrosine protein phosphatase 1B (PTP-1B) and phosphorylation of serine residue of IRS-1, which are negatively associated with insulin sensitivity (Chuang et al. 2011).

Trans-resveratrol and quercetin, two polyphenols present in grapes, exert antidiabetic properties by activating adenosine monophosphate kinase (AMPK) path-

ways in differentiated myoblasts and preadipocytes (3T3-L1), respectively (Park et al. 2007; Ahn et al. 2008). The AMPK pathway is important in controlling cellular energy homeostasis, and its activation leads to the generation of energy through glycolysis and fatty acid oxidation (Rupasinghe et al. 2016). Furthermore, AMPK activation suppresses gluconeogenesis and lipogenesis (Hwang et al. 2009). The overall effect of AMPK activation favors the improvement of T2D-related metabolic complications. Treatment of 3T3-L1 cells with quercetin increases phosphorylation of AMPK and acetyl Co-A carboxylase (ACC), resulting in activation of AMPK and inactivation of ACC, a key enzyme involved in fatty acid biosynthesis that is phosphorylated and inactivated by phosphorylated AMPK. The inactivation of ACC decreases fatty acid synthesis and reciprocally increases fatty acid oxidation. Indeed, quercetin decreases adipogenesis and lipid accumulation in 3T3-L1 adipocytes in a dose-dependent manner. In addition, quercetin decreases adipogenic transcription factors and adipogenetic enzyme expression in 3T3-L1 adipocytes (Ahn et al. 2008). The increase of fat oxidation is associated with the improvement of insulin sensitivity and peripheral glucose disposal. Naringenin, a prominent polyphenol in citrus fruits and tomatoes, is another example, which increases glucose uptake in L6 rat myotubes by activating the AMPK pathway (Zygmunt et al. 2010).

Glucose is absorbed from the intestine and reabsorbed in kidneys through renal filtrate by sodium-dependent glucose transporters (SGLT1 and SGLT2), making these transporters therapeutic targets for antidiabetic drugs. Apples are rich in phlorizin or phloridzin, a glycoside of phloretin of the dihydrochalcone group (Matsuda et al. 2003), which inhibits SGLT2 in COS-7 cells (Pajor et al. 2008). The glucose moiety of the phlorizin binds to the glucose-binding site of SGLT2, thereby inhibiting glucose transport (Pajor et al. 2008). However, phlorizin is deglycosylated in the intestine before absorption. Thus, the low bioavailability of this molecule limits its use as an SGLT2 inhibitor in vivo (Li et al. 2006). Naringin is another flavonoid that inhibits glucose uptake in both intestinal and renal brush border membranes. However, the efficacy of naringin is lower than phlorizin (Li et al. 2006). Antidiabetic effects of different fruit extracts and their polyphenols assessed in cell model systems seem to vary in animal models or human clinical trials. The metabolism and low bioavailability of polyphenols largely explain their low in vivo bioactivity and efficacy (Manach et al. 2004). More recently, it has been reported that delphinidin-3-*O*-runtinoisde, an anthocyanin present in berries, can induce GLP-1 secretion in murine GLUTag cell model (Kato et al. 2015).

17.3.3 In vivo Antidiabetic Effects of Fruit Polyphenols

The data of animal models demonstrate that the fruit phenolic compounds possess antidiabetic effects through several different mechanisms. Table 17.3 shows studies that were conducted in mouse and rat research models to evaluate the effect of polyphenol-rich fruit extracts for the treatment of T2D and related complications.

Table 17.3 Antidiabetic properties of fruit extracts and polyphenols in animal models

Fruit extract/ phenolics	Biological property	Mechanism of action	Animal model	References
Blueberry	Improve insulin sensitivity and hyperglycemia	Activate AMPK pathway	KK-A^{y}mice	Takikawa et al. (2010)
	Improve insulin sensitivity and reduce adipose tissue inflammation	Attenuate inflammatory genes in adipose tissue	C57BL/6 mice	DeFuria et al. (2009)
	Hypoglycemic activity		C57BL/6 mice	Grace et al. (2009)
Black chokeberry	Inhibit glucose uptake and improve antioxidant status	Inhibit maltase and sucrase activities and reduce lipid peroxidation	Wistar rats	Jurgoński et al. (2008)
Guava peel and seed extracts	Hypolipidemic activity	Reduce triglycerides, total cholesterol, LDL and VLDL, and increase HDL	Wistar rats	Rai et al. (2010)
Jambul fruits	Antioxidant and hypoglycemic effects	Reduce ROS generation, blood, and hepatic glucose, upregulate insulin, GLUT2, and glucokinase	Swiss albino mice	Samadder et al. (2011)
Acerola fruits	Hypoglycemic effect	Inhibit α-glucosidase activity and glucose transporter expressions	ICR mice	Hanamura et al. (2006)
Quercetin	Modulate adiponectin secretion	–	Wistar rats	Wein et al. (2010)
Naringenin	Reduced insulin resistance, Reduced inflammation Reduced hyperlipidemia	Activate AMPK pathway Decrease blood TNF-α and leptin Decrease serum and hepatic cholesterol	C57BL/6 mice	Pu et al. (2012)
Resveratrol	Improve hyperinsulinemia and dyslipidemia	Activate AMPK pathway	Obese Zucker rats	Rivera et al. (2008)
Myricetin	Improve insulin sensitivity	Increase IRS-1 and P13K expressions and phosphorylation of IR, IRS-1, and Akt	Wistar rats	Liu et al. (2007)
Cyanidin-3-*O*-glucoside	Improve hyperglycemia and insulin resistance	Reduce the gene expression of retinol-binding protein	KK-A^{y}mice	Sasaki et al. (2007)

A number of berry extracts and their isolated polyphenols have been investigated. These extracts were mainly derived from berry fruits, tropical fruits, and traditional fruits (Basu et al. 2009; Rai et al. 2010; Takikawa et al. 2010; Samadder et al. 2011). Several animal studies report that blueberries protect against insulin resistance and hyperglycemia (Takikawa et al. 2010; DeFuria et al. 2009). Blueberry extracts, when provided at a dose of 375 mg anthocyanins/kg body weight, improved insulin sensitivity and hyperglycemia in KK-A^{γ} mice, a mice model of type 2 diabetes (Takikawa et al. 2010), possibly by activating AMPK. Blueberry extracts were shown to be protective against adipose tissue inflammation and insulin resistance induced by high-fat feeding in male C57BL/6 mice and downregulated the expression of inflammation-related genes (DeFuria et al. 2009). The crude blueberry extract and anthocyanin-rich blueberry extract were compared for their antihyperglycemic activity in C57BL/6 mice. It was found that although both extracts reduced blood glucose levels, the anthocyanin-rich extract showed a greater hypoglycemic effect than the crude extract (DeFuria et al. 2009). A further study demonstrated that delphinidin-3-*O*-glucoside plays a prominent role in the observed greater bioactivity in the anthocyanin-rich extract (Grace et al. 2009). Purified berry anthocyanins rather than berry extracts reduce serum leptin, triacylglycerols, and cholesterol levels in mice fed a high-fat diet (Prior et al. 2009). The reasons for the observed differences are not fully understood. The presence of different types, quantities, and configuration of anthocyanins, amounts of other non-phenolic constituents, total consumption, and bioavailability were thought to be possible contributory factors (Prior et al. 2009). In comparison to fresh fruit extracts, blueberry juice does not show hypoglycemic effects in diabetic mice fed either a low-fat or a high-fat diet. However, the juice-fed mice have decreased epididymal and retroperitoneal fats, which is noteworthy for its potential use as an anti-obesity and antidiabetic agent in the long term (Prior et al. 2010).

The antidiabetic effects of strawberry, chokeberry (genus *Aronia*), Chinese bayberry (*Myrica rubra* L.), and elderberry (genus *Sambucus*) extracts have also been investigated. It is reported that women with metabolic syndrome decreased lipid peroxidation and blood glucose levels after four weeks of treatment with freeze-dried strawberry powder (Basu et al. 2009). Chokeberry extracts improved the antioxidant status and inhibited the activity of carbohydrate-hydrolyzing enzymes in mouse models of metabolic syndrome (Jurgoński et al. 2008). Anthocyanins from Chinese bayberry extracts protected pancreatic β-cells from oxidative stress, necrosis, and apoptosis (Zhang et al. 2011). Guava (genus *Psidium*) and jambul (*Syzygium cumini* L.) are fruits grown in tropics. Raw fruit peel of guava and seed extracts of jambul were identified as potential hypoglycemic and hyperlipidemic agents (Rai et al. 2010; Samadder et al. 2011). Acerola (*Malpighia emarginata* L.) fruit extracts are well known for their high vitamin C and carotenoid contents. Furthermore, acerola is a rich source of polyphenols. Polyphenol-rich fractions of acerola prevent postprandial hyperglycemia in an experimental model of gene knockout mice (Hanamura et al. 2006). Although some studies have examined the antidiabetic properties of phenolic compounds present in fruits and vegetables, their mechanisms of action are not well understood.

The antidiabetic properties of phenolic compounds are partially achieved via the activation of AMPK-mediated signaling pathways. Supplementation of naringin in

rats fed a high-fat diet protects against developing metabolic syndrome by activating the AMPK signaling pathway (Pu et al. 2012). Similarly, oral administration of resveratrol to obese Zucker rats reduced hyperinsulinemia and dyslipidemia while increasing the activity of AMPK in the liver and visceral adipose tissues (Rivera et al. 2008). When pigs were fed low doses of resveratrol for nine months, the expression of genes related to lipid metabolism and metabolic disorders were altered (Azorín-ortuño et al. 2011). Quercetin modulates adiponectin secretion in male Wistar rats through a PPAR-γ independent pathway (Wein et al. 2010). Myricetin improves insulin sensitivity in male Wistar rats fed a high-fructose diet through insulin action on insulin substrate-1 associated PI3K and GLUT4 activity (Liu et al. 2007). Anthocyanin, cyanidin-3-*O*-glucoside, ameliorates hyperglycemia and insulin resistance by reducing the gene expression of retinol-binding protein 4 in diabetic mice (Sasaki et al. 2007).

17.4 Fruit and Vegetable Intake and Risk of Type 2 Diabetes

Most epidemiological and clinical studies to date indicate that intake of fruits, vegetables, polyphenol-rich foods or isolated fruit phenolic compounds have antidiabetic effects.

17.4.1 Epidemiological Studies

Evidence from epidemiological studies suggests that consumption of fruits and vegetables is associated with a reduced risk of developing chronic diseases such as diabetes, CVDs and cancer (Martínez-González 2011). The European Prospective Investigation of Cancer (EPIC)-Norfolk study investigated the association between fruit and vegetable intake and T2D (Harding et al. 2008). The study included men and women of 40–75 years old from Norfolk, England, and followed up for 12 years. The results revealed that fruit and vegetable consumption is positively associated with plasma vitamin C levels and negatively associated with the incidence of T2D. The association between the intake of fruits, vegetables, and fruit juices and the risk of T2D was also assessed in the Nurses' Health Study (Bazzano et al. 2008). Participants in this study were female nurses from different states of the USA, aged between 38-63 years, and who were free from CVDs, cancer, or diabetes. This study showed that while the increased consumption of whole fruits and green leafy vegetables reduced diabetes risk, the number of servings of fruits and vegetables did not affect diabetes risk. The Finnish Mobile Clinic Health Examination Survey was a cohort study to examine the effect of different foods on the incidence of T2D. The results indicated that high intakes of green vegetables, berries, and fruits were associated with a lower risk of T2D (Montonen et al. 2005). Similarly, women eating more than one apple a day showed a 28% reduction of risk for developing T2D compared to those eating less than one apple a day (Song et al. 2005). However, the intake of fruit juices showed a different effect from

consumption of whole fruits and vegetables. In a prospective cohort study, Chinese men and women in Singapore, aged 45–74 years and who were free of diabetes and CVD, consumed soft drinks and juices more than twice a week had a higher risk of developing T2D (Odegaard et al. 2010).

The results of the published studies are inconsistent. While several studies showed beneficial effects of consumption of fruits and vegetables, other studies reported no effects. Two perspective cohort studies concluded that the consumption of fruits is not associated with diabetes risk (Harmer and Chida 2007; Villegas et al. 2008). A meta-analysis including six prospective cohort studies focused on the relationship between fruit and vegetable consumption and the risk of T2D concluded that except for green leafy vegetables, other vegetables either alone or in combination had no effect on the risk of T2D (Carter et al. 2010). The dietary intake of fruits and vegetables in Chinese women was assessed in the Shanghai Women's Health Study (SWHS). It was found that vegetable intake but not fruit intake reduced the incidence of T2D. It is suggested that vegetables are more beneficial than fruits in preventing diabetes. The authors have speculated that high amounts of fructose and other sugars in fruits may interfere with the beneficial biological activities of vitamins, minerals, and phytochemicals such as polyphenols on glucose metabolism and homeostasis. It also should be noted that in human studies, many factors such as lifestyle behaviours, diets, exercise, supplements, drugs, etc. might not have been well controlled, which affect to a certain extent the benefits of fruits and vegetables (Villegas et al. 2008). The incorporation of fruits and vegetables into regular diets is recommended. Canada's Food Guide (2007) recommends eight to ten servings a day of fruits and vegetables. However, the prevention of T2D by fruits is not well established, and further investigations through well-designed clinical studies are warranted.

17.4.2 Human Clinical Studies

There are limited human clinical studies examining the benefits of dietary intervention with fruits on T2D. A double-blind placebo-controlled clinical trial was conducted in postmenopausal women with T2D for one year, where patients consumed flavonoid-enriched chocolates (containing 850 mg flavan-3-ols and 90 mg epicatechin) and 100 mg isoflavones or matched placebo. A significant improvement in the peripheral insulin resistance, blood total HDL cholesterol ratio, and LDL levels was observed compared to the control group (Curtis et al. 2012). However, the Women's Health (Song et al. 2005) and Iowa Women's Health (Nettleton et al. 2006) studies showed that dietary intake of flavonoids did not reduce T2D incidence. However, the Women's Health Study emphasized the importance of consuming apples in reducing the risk of T2D.

The Netherlands Prospective Cohort Study (NLCS) assessed the effects of dietary intake of flavonoids on weight gain, which is related to the development of obesity and T2D (Curtis et al. 2012). This study reported that women who had high intakes of flavonoid-rich foods over 14 years showed significantly lower body mass index compared to those who had low consumptions of flavonoids. It was concluded

that dietary intake of flavones, flavonols, and catechins are beneficial to women for controlling body weight (Hughes et al. 2008). Although not examined in this study, the lower body mass index in women consuming fruit flavonoids is considered to be beneficial to the prevention of diabetes since obesity increases the risk of T2D. Nevertheless, the evidence supporting the beneficial effects of fruit polyphenols on diabetes is inconsistent, and more human clinical investigations are required.

17.5 Conclusions

There is substantial evidence that fruit and vegetable intake reduces the risk of T2D (Li et al. 2014; Wang et al. 2016). Fruit extracts rich in polyphenols have shown beneficial effects in controlling the pathogenesis of T2D in cellular-based models and animal models. Most fruit extracts and constituent polyphenols inhibited the activity of enzymes involved in carbohydrate hydrolyzation, digestion, and protein glycation. The presence and position of hydroxyl groups in polyphenols (especially flavonoids) are important to their bioactivity. Activation of AMPK by fruit polyphenols is a shared mechanism in most studies while other mechanisms are also reported.

The results of epidemiological studies on the association between the consumption of fruits and the risk of T2D are controversial. The contradictory effects of whole fruits or fruit juice may be a result of interference of sugars contained in fruits with glucose homeostasis or a contribution of fruit sugar to the body sugar pool. Therefore, future human clinical trials should evaluate whole fruits and sugar-free fruit polyphenols. Emerging evidence suggests that berry and citrus fruit extracts and fruit polyphenols such as resveratrol, naringenin, anthocyanins, quercetin, proanthocyanidins, and phlorizin are promising natural phytochemicals for preventing and managing T2D and the associated complications. Precaution should be taken by diabetic patients or those with a high risk for developing diabetes when taking fruits and fruit juices, especially those with a high sugar content.

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Chapter 18
Antioxidants in the Prevention and Treatment of Liver Diseases

Sha Li, Ning Wang, Yi-Gang Feng, Hong-Yun Li, and Yibin Feng

Abstract Oxidative stress is believed to play a role in the initiation and progression of liver diseases, leading to the proposal that antioxidant therapy has the potential to prevent and treat liver diseases that involve oxidative stress. This chapter reviews preclinical studies using animal models that evaluate the efficacy of various antioxidants including pure compounds and herbal medicines. Furthermore, therapeutic outcomes of antioxidants in patients with alcoholic liver disease and nonalcoholic liver disease are also summarized. Although a great deal of encouraging data on various antioxidants has been obtained in animal studies, the potential of application of antioxidants solely or as adjuvant therapy in human liver diseases is still controversial and challenging. On the one hand, this might be partly due to the fact that only the early phases of liver diseases are studied in most animal models, suggesting that antioxidants might have a greater role in less advanced hepatic diseases. On the other hand, translational research should also be further improved to realize the application of antioxidants in liver diseases. Factors such as the duration of treatment, dose to be used, bioavailability in human, and mode of administration should be carefully explored in future studies. Additionally, study design, clinical endpoints, and choice of patient population should also be critically considered in clinical trials. In summary, intensive efforts should be made to establish a role for antioxidant treatment of liver disease.

Keywords Antioxidants • Oxidative stress • Lipid peroxidation • Liver diseases

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18.1 Introduction

Free oxygen radicals and other reactive oxygen species (ROS) are generated during oxygen metabolism in biological systems. The liver is an important site of free radical production by hepatic enzymes. The mitochondria, endoplasmic reticulum, and peroxisomes of hepatic tissues generate ROS (Webb and Twedt 2008; Zhu et al. 2012). Under some conditions, free radicals are essential for signal transduction and gene expression and can have beneficial roles (Videla 2009). However, they become detrimental when the levels of superoxide production are increased by the activity of the electron transport chain. The imbalance between ROS production and antioxidants defenses induces oxidative/nitrosative stress in the body, which can initiate lipid peroxidation, trigger DNA injury, oxidize molecules in tissues, and more importantly, modulate cell signaling transduction processes; these changes lead to cellular and tissue damage (Li et al. 2015). Since the liver is particularly sensitive and susceptible to oxidative stress, ROS constitutes a crucial background of many hepatic disorders, and contributes to development of metabolic, inflammatory, and proliferative liver diseases. In fact, liver diseases are always characterized by augmented oxidative stress, which can also trigger hepatic injury (Horie et al. 2006). In addition to high levels of oxidative stress, multiple studies have shown that the extent of lipid peroxidation and oxidative protein always correlates with injury severity, which is further related to the progression of many liver diseases (Videla 2009).

Enzymatic and nonenzymatic systems control oxidative stress and are essential for maintaining cellular redox homeostasis under physiological conditions (Li et al. 2015) (Fig. 18.1). Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR), as well as nonenzymatic particles of electron acceptors such as glutathione (GSH), vitamin C, and vitamin E are of great importance in the cellular response to oxidative stress. In hepatic injury induced by metabolic disorders or hepatotoxins, the decreased activity of these antioxidant enzymes and a reduction of electron acceptors, such as GSH, occur frequently. Increases in oxidative stress regulate the activity of redox-sensitive transcription factors such as nuclear factor κ B (NF-κB), activator protein-1 (AP-1), and early growth response protein 1 (Egr-1). Importantly, a distinctive defense mechanism to eliminate ROS exists in the liver with the involvement of nuclear factor E2-related factor 2 (Nrf2). Increased oxidative stress activates cytoplasmic Nrf2, which then inhibits mitochondrial injury induced by oxidative stress by increasing the expression of antioxidant enzymes, maintaining the mitochondrial redox state, protecting against opening of mitochondrial permeability transition pore, and enhancing mitochondrial biogenesis (Wu et al. 2012; Li et al. 2015). However, when cellular protective defenses fail to remove ROS and reactive nitrogen species (RNS), the resultant increased oxidative stress alters mitochondrial function, modifies immune responses, regulates cytokine expression, and stimulates signaling cascades that result in apoptosis or cellular and tissue damage in the liver (Singal et al. 2011).

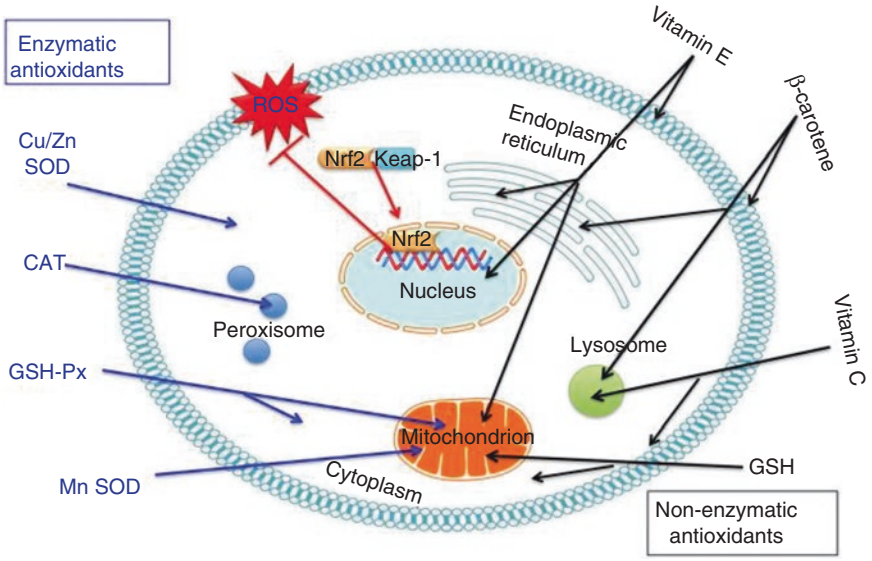

Fig. 18.1 Cellular antioxidant defenses including enzymatic and nonenzymatic systems and Nrf-2 activation

Antioxidants have been studied for the prevention and treatment of liver diseases. Generally, antioxidants are molecules that can donate electrons to free radicals (Fig. 18.2). However, substances that activate and/or enhance antioxidant defense in vivo are also sometimes regarded as antioxidants. Many compounds possessing outstanding antioxidative property have been used to prevent and treat liver diseases in experimental animal studies, including those from plant- or food-derived natural compounds (Li et al. 2007, 2013, 2014a; Guo et al. 2012). A beneficial role of natural or synthesized antioxidants in liver diseases in animal studies is likely to increase enthusiasm for use in patients with liver diseases. However, the therapeutic efficacy of antioxidants in various liver diseases is unclear in clinical trials (Singal et al. 2011). For example, vitamin E therapy in nonalcoholic steatohepatitis (NASH) shows some promising results as an antioxidant therapy for acute alcoholic hepatitis (Sanyal et al. 2010; Bell et al. 2012). In contrast, although oxidative stress is suggested to play a role in chronic viral hepatitis, there is as yet no convincing evidence showing that antioxidants are beneficial in the treatment of chronic patients with hepatitis C and hepatitis B (Acar et al. 2009, Gomez et al. 2010, Farias et al. 2012, Tasdelen Fisgin et al. 2012). Some explanations for this may be related to difficulty in understanding the detailed mechanisms of action of antioxidants and also challenges associated with the design and implementation of translational research and clinical trials. This chapter reviews the use of various antioxidants in a broad spectrum of liver diseases from data obtained from in vitro and in vivo studies and discusses the current status

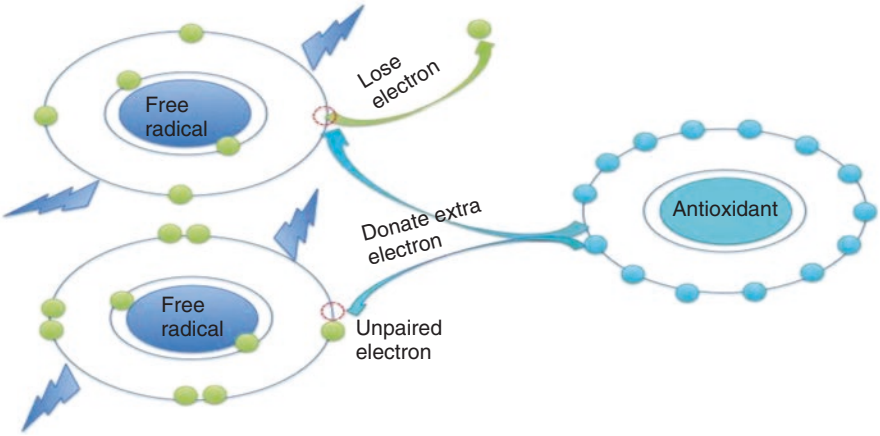

Fig. 18.2 Antioxidants scavenge free radicals by donating an extra electron

of antioxidant use in treating liver diseases including chronic viral hepatitis, alcoholic liver diseases, and NASH. Furthermore, drawbacks and challenges as well as perspective for the future use of antioxidants therapy in liver diseases are also discussed.

18.2 Antioxidants in the Prevention and Treatment of Liver Diseases

18.2.1 Alcoholic Liver Diseases

The common features of excessive alcohol exposure are often characterized by ROS production, mitochondrial damage, and hepatic steatosis. After alcohol exposure, increased ROS production and reduction of antioxidants activity occur in cytosol, mitochondria, and endoplasmic reticulum (Zima and Kalousova 2005). Dehydrogenase systems and microsomal ethanol-oxidizing systems (MEOS) are major enzymatic pathways responsible for ethanol metabolism. In the oxidation processes of alcohol with dehydrogenase and microsomal ethanol-oxidizing system (MEOS), substantial increases in nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADP+) occur, leading to the generation of ROS and oxidative stress (Panchenko et al. 2013; Wu and Cederbaum 2009; De Minicis and Brenner 2008). For impaired antioxidant defenses, GSH may be the most important nonenzymatic antioxidant that is affected in alcoholic liver diseases (De Minicis and Brenner 2008). Alcohol depletes GSH in mitochondria of centrilobular hepatocytes, and this precedes the development of mitochondrial injury and lipid alterations (García-Ruiz et al. 1994). Regarding antioxidant enzymes, a striking decrease in protein levels and activities of SOD, CAT, and GPX

have been detected in animals challenged with ethanol (Zima and Kalousova 2005). The alteration of enzyme activities and oxidative stress are positively correlated with the severity of lipid peroxidation and liver damage.

The underlying pathways of oxidative stress-caused alcoholic liver injury have been studied in some detail. Under peroxidative conditions, the mitogenactivating protein kinase (MAPK) pathway is stimulated by the activation of protein kinase C (PKC) or degradation of protein phosphatases (Bhalla et al. 2002; Kamata et al. 2005; Han et al. 2016). Disruption of this signaling network ultimately leads to steatosis and hepatic inflammation. On the one hand, activated MAPK leads to stimulation of the Bax/Bcl2 pathway, resulting in hepatocyte death. On the other hand, signaling by MAPK induces the protective activation of the Keap1-Nrf2 pathway, which then interacts with the antioxidant response element (ARE) to stimulate the expression of antioxidant enzymes such as SOD and CAT (Zima and Kalousova 2005; Li et al. 2015). Therefore, substances that normalize MAPK and/or activate Keap1-Nrf2 are potential candidates to prevent and treat alcoholic liver disease (ALD).

With better demonstration of the underlying mechanisms of oxidative stress in ALD, antioxidant therapy has been considered to prevent or treat ALD in in vitro and animal studies. Many foods and plants, such as fruits and medicinal plants rich in natural antioxidants, were used to eliminate ROS/RNS and to protect the liver from oxidative stress (Li et al. 2015). In recent years, a number of plant products have been used to alleviate liver injury induced by alcohol in animal models. For example, betaine, catechin, quercetin, and epigallocatechin gallate (EGCG) are protective against alcohol-caused oxidative stress in HepaG2 hepatic cells; these agents all downregulated GPX4 expression, while quercetin, catechin, and betaine prevented the formation of malondialdehyde (MDA)/4-hydroxynonenal (4HNE) induced by ethanol. In addition, catechin reduced the induction of CYP2E1, and betaine attenuated the upregulation of heat shock protein 70 by ethanol (Oliva et al. 2011).

The effect of Korean red ginseng in diminishing oxidative stress and steatosis induced by alcohol in a murine model and ethanol-treated hepatocytes has been investigated. The results indicated that RGE reduces the induction of cytochrome P4502E1, 4-HNE, and nitrotyrosine levels caused by alcohol. More importantly, red ginseng restores the phosphorylation of 5′-adenosine monophosphate-activated protein kinase (AMPK) that is decreased by alcohol. Additionally, RGE significantly inhibited fat accumulation in hepatocytes treated with alcohol by decreasing sterol regulatory element-binding protein-1 and increasing the expression of sirtuin 1 (Sirt 1) and peroxisome proliferator-activated receptor-α. This study suggested that RGE can potentially treat ALD by activating the AMPK/Sirt1 pathway (Han et al. 2015). Another study demonstrated that demethyleneberberine (DMB), a natural mitochondria-targeting antioxidant, penetrates mitochondrial membranes and accumulates in mitochondria, thus ameliorating oxidative stress induced by acute alcohol intake (Zhang et al. 2015). In chronic ethanol-treated mice, DMB ameliorated lipid peroxidation and macrosteatosis by suppression of CYP2E1 and normalization of Sirt 1/AMPK pathway-related fatty acid oxidation. Moreover, MitoQ, a synthetic mitochondria-targeted antioxidant, also has protective effects in a mouse model of ALD (Zhang et al. 2015). Green tea, which is rich

in water-soluble antioxidants, also has beneficial effects on the antioxidant defenses in the liver of rats chronically consuming ethanol (Augustyniak et al. 2005). Reductions of antioxidant levels, and increases in lipid peroxidation and protein modifications caused by ethanol are partially normalized by green tea. Epicatechin and epicatechin gallate are thought to be responsible for antioxidative activity of green tea.

In addition to polyphenol and flavonoids compounds, the antioxidant property of polysaccharide has also been investigated. Non-starch polysaccharide derived from peduncles of *Hovenia dulcis* has a protective effect in mice with acute alcoholic liver injury by enhancing the expression and activity of SOD as well as GPX (Wang et al. 2012a). Treatment of rats with polysaccharide from *Lycium barbarum* restored MDA levels and improved antioxidant defense in the liver, which effectively alleviated liver damage and prevented the progression of fatty liver (Cheng and Kong 2011). The effects of antioxidants (including natural products and synthesized compounds) on alcoholic liver injury are summarized in Table 18.1 (adopted from Li et al. 2015). As seen from Table 18.1, antioxidant therapy is a promising strategy for the prevention of alcoholic liver injury in animal studies. However, the active ingredients in natural plants that lead to reduced oxidative stress in these studies are thought to be flavonoids, and the underlying mechanisms have yet to be fully investigated.

18.2.2 Nonalcoholic Liver Diseases

Nonalcoholic fatty liver disease (NAFLD), an indicator of metabolic syndrome, is characterized by abnormal fatty acid deposition and is becoming increasingly prevalent (Li et al. 2015). NASH, a more advance form of NAFLD, is generally described by the occurrence of steatosis with severe inflammation and progressive fibrosis (Koek et al. 2011; Mitsuyoshi et al. 2006). Oxidative stress is considered to be a pivotal factor during the development of this disease. During free fatty acid metabolism in mitochondria, microsomes, and peroxisomes, ROS are generated in bulk. Unfortunately, antioxidant defenses are insufficient to combat the effects of excessive ROS production. An inflammatory cascade involving cytokines is triggered by oxidative stress (Koek et al. 2011; Takaki et al. 2013). When a free radical accepts an electron from an unsaturated fatty acid, lipid peroxidation is launched, and this then triggers a chain reaction creating lipid peroxides, resulting in membrane dysfunction and the generation of reactive metabolites such as MDA and 4-HNE (Koek et al. 2011). Oxidative stress affects hepatic lipid metabolism at multiple levels, ranging from simple lipid storage to inflammation, a process referred to as a "secondary hit." Necro-inflammation, resembling inflammation with rapid necrosis of tissue, is induced by nuclear and protein dysfunction as well as mitochondrial DNA damage in hepatocytes (Mitsuyoshi et al. 2006).

Antioxidants from medicinal plants and pure compounds have been intensively studied in NAFLD. As a preventive treatment of NAFLD, they are often used during

Table 18.1 The effects of natural products on alcoholic liver damage

Models (prevention/treatment)	Materials	Effect	Bioactive compounds	References
Mice treated with alcohol (treatment)	Freeze-dried, germinated, and fermented mung bean	↑ Antioxidant levels, NO		Mohd Ali et al. (2013)
Mice treated with alcohol (treatment)	Korean red ginseng	AMPK/Sirt1 activation	Ginsenoside components	Han et al. (2015)
Rats treated with ethanol diet (prevention)	Green tea	↑ Enzymes, nonenzymatic antioxidants; ↓ lipid and protein oxidation	Epicatechin, epicatechin gallate	Augustyniak et al. (2005)
Rats treated with ethanol (prevention)	*Ziziphus mauritiana* leaf	↓ ALT, AST, ALP, total bilirubin, CAT; ↑ GSH-Px, glutathione reductase, and SOD	Tannins, saponins, and phenolic compounds	Dahiru and Obidoa (2007)
Rats exposed to ethanol (prevention)	*Amaranthus hypochondriacus* seed	↓ MDA, NADPH; ↑ Cu, Zn-SOD	Total phenols	Lopez et al. (2011)
Rats treated with ethanol (prevention)	Methanolic extract from *Hammada scoparia* leaves	↓ Aminotransferase, glycogen synthase kinase-3 beta, lipid peroxidation; ↑ GSH-Px	Phenolic compounds	Bourogaa et al. (2013)
Mice chronically treated with alcohol (prevention)	Jujube honey	↓ Lipoprotein oxidation, AST, ALT, MAD, 8-hydroxy-2-deoxyguanosine; ↑ GSH-Px	Phenolic acids	Cheng et al. (2014)
Rats chronically treated with ethanol (prevention)	Virgin olive oil	↓ Transaminases levels, hepatic lipid peroxidation; ↑ GSH-Px, SOD, and CAT	Tocopherols, chlorophyll, total polyphenols	Kasdallah-Grissa et al. (2008)
Mice acutely treated with alcohol (prevention)	Peduncles of *Hovenia dulcis*	↓ ALT, AST, MDA; ↑ SOD, GSH-Px	Non-starch polysaccharide	Wang et al. (2012a)
Rats chronically treated with ethanol	*Lycium barbarum*	↓ ALT, AST; SOD, ↑ CAT, GSH-Px and GSH	Polysaccharide	Cheng and Kong (2011)

ALP alkaline phosphatase, ↑ means increase or enhance, ↓ means decrease

the early phases of the development of the disease. Both in vitro and animal studies using models of obesity, such as a high-fat diet or a methionine- and choline-deficient diet (MCDD), reveal that a variety of substances reduce oxidative stress and thus alleviate NAFLD. In in vitro models using free fatty acid-induced lipid overload and oxidative stress in hepatocytes and Kupffer cells, isoquercitrin (IQ), a flavonoid, weakened lipid overload and ROS production in hepatocytes via the AMPK pathway (Hassan et al. 2014). In models of NAFLD triggered by high-fat diet or obesity, a polyphenol extract from brown algae *Ecklonia cava* (a seaweed with a rich polyphenolic content) improved hepatic lipogenesis, oxidative stress, and inflammation via activation AMPK and Sirt 1 (Eo et al. 2015), and EGCG diminishes oxidative stress, inflammation, and fibrosis via pathways such as transforming growth factor-β (TGF-β)/SMAD and NF-κB (Xiao et al. 2014). Liraglutide, a glucagon-like peptide-1 (GLP-1) analogue, has antioxidant and hepatoprotective properties, which might be regulated by the elevation of adiponectin levels and the inactivation of Jun N-terminal kinase-signaling (JNK) (Gao et al. 2015). Garlic essential oil, choline, and fructooligosaccharide (FOS, used as a sweetener) also protect against NAFLD through regulation of lipid metabolism and oxidative stress (Lai et al. 2014; Xiao et al. 2014).

In models of NASH induced by MCDD diet, antioxidants, such as indole-derived NecroX-7 (a mitochondrial-specific ROS inhibitor), alpha-lipoic acid, and 6-gingerol (active constituent of fresh ginger), also showed beneficial effects via suppression of oxidative stress and inflammatory response. Specifically, indole-derived NecroX-7 suppressed whole-cell ROS/RNS (Chung et al. 2015); alpha-lipoic acid improved antioxidative capacity of liver by increasing SOD activity and GSH levels (Stanković et al. 2014); 6-gingerol downregulated cytochrome CYP2E1 and JNK (Tzeng et al. 2015). Additionally, retinoic acid-related orphan receptor alpha (ROR alpha) can regulate diverse genes related to lipid metabolism and which have been found to have reduced expression in the livers of patients with NASH. It also induces the expression of SOD2 and GSX and thus protects mice from NASH induced by MCDD (Han et al. 2014). Studies using high-fat diets or streptozotocin-induced diabetic model to investigate the efficacy of antioxidant substances in NAFLD are summarized in Table 18.2 (adopted from Li et al. 2015). In Table 18.2, most of these compounds or plants show both antioxidant and hepatoprotective effects. Regarding the streptozotocin-induced diabetic model, many antioxidants displayed positive roles not only for the prevention but also for the treatment of hyperglycemia, further implying a promising potential role for antioxidants.

18.2.3 Liver Diseases Induced by Pharmaceuticals and Pollutants

Since the liver is a central organ for detoxification and metabolism, it is vulnerable to damage by pharmaceuticals and pollutants (Li et al. 2015), including paracetamol, D-galactosamine, lipopolysaccharide (LPS), heavy metals, microcystin, and carbon tetrachloride (CCl_4). The production of ROS/RNS and the reduction of antioxidant

Table 18.2 The effects of some antioxidants/plants on NAFLD in animal studies

Models (prevention/treatment)	Antioxidant/plants	Effects	References
Streptozotocin-induced diabetic rats (treatment)	Stobadine		Cumaoglu et al. (2007)
Mice fed with high-fat diet (prevention and treatment)	*Moringa oleifera* leaves; haw pectic oligosaccharide; *Thymbra spicata*	↑ GSH; ↓ ALT, AST, ALP, lipid peroxidation	Li et al. (2014b); Akkol et al. (2009); Das et al. (2012)
Streptozotocin-induced diabetic rats (treatment)	Berberine; N-acetylcysteine; *Oroxylum indicum* stem bark; maslinic acid; resveratrol	Antioxidation	Zhou and Zhou (2011)
Diabetic rats fed on a high-fat thermolyzed diet (prevention)	Omega-3 polyunsaturated fatty acids	↑ SOD, CAT; ↓ triglycerides, non-esterified fatty acid, lipoperoxidation	de Assis et al. (2012)
Liver damage in diet-induced atherosclerotic rats (prevention)	*Tulbaghia violacea* rhizomes	↓ LDH, AST, ALT, ALP, bilirubin antioxidation	Olorunnisola et al. (2012)
Rabbits with high-fat diet (prevention)	Apolipoprotein A-I	↑ SOD, GSH-Px; ↓ iNOS, MDA	Wang et al. (2013)
Rats fed a high-fat diet (prevention)	Black cabbage sprout	↑ SOD, CAT, NADPH, GSH-Px, GRD GST	Melega et al. (2013)
Streptozotocin-induced diabetic aged rats (prevention)	Vitamins C and E	Antioxidation, hepatoprotection	Naziroglu et al. (2011)
Streptozotocin-induced diabetic rats (prevention)	Acai; *Herba bidenti*; (−)-epicatechin; *Stevia rebaudiana*; *Aloe vera* leaves	Antioxidation, hepatoprotection	Guerra et al. (2011)
Streptozotocin-induced diabetic mice (prevention)	*Terminalia glaucescens* leaves	Antioxidation	Njomen et al. (2008)

LDH lactate dehydrogenase, *NADPH* nicotinamide adenine dinucleotide phosphate-oxidase, *iNOS* inducible nitric oxide synthase (iNOS), *GRD* glutathione reductase, *GST* glutathione S-transferase, ↑ means increase or enhance, ↓ means decrease

defenses have been suggested as indicators of the hepatotoxic potential of these substances (Videla 2009). Elevated markers of oxidative stress and lipid peroxidation and reduction of antioxidants in the liver occur after administration of many pharmaceuticals. For example, sulfasalazine, a drug used to treat inflammatory bowel diseases, is thought to cause liver damage via oxidative stress (Linares et al. 2009). Hepatic disorders induced by paracetamol are related to increases in MDA and significant decreases of SOD activity (Mladenovic et al. 2009). Mercury chloride causes liver damage in rats with simultaneous decreases in SOD activity and reduced activities of CAT, GPX, GR, and glucose-6-phosphate dehydrogenase (G6PD) (Bando et al. 2005).

A variety of antioxidants relieve liver damage induced by hepatotoxic substances and are summarized in Table 18.3 (adopted from Li et al. 2015). Animals treated with CCl_4, a CC chemokine, have been extensively used to study the role of antioxidants in hepatotoxicity. Exposure to CCl_4 increases oxidative stress and leads to lipid peroxidation and damages hepatocellular membranes. This is followed by secretion of pro-inflammatory cytokines that ultimately results in hepatic injury. A number of natural products, especially herbal plants, have been used to treat liver dysfunction caused by CCl_4. For example, *Coptidis rhizome* and its bioactive compound berberine, a medicinal plant widely used in Chinese Medicine, exert beneficial effects on CCl_4-induced hepatotoxicity in rats partly by reducing phosphorylation of extracellular-signal-regulated kinases (Erk1/2) expression (Ye et al. 2009; Feng et al. 2011; Wang et al. 2012b). In addition, substances, such as anthocyanins, present in all tissues of higher plants like the root of Radix Platycodi activate Nrf2 to protect cells from oxidative stress through upregulation of antioxidant gene expression in dimethylnitrosamine- or cadmium-induced hepatic injury models (Niture et al. 2009). While many natural products act as antioxidant and hepatoprotective agents (see Table 18.3), their mechanisms are still unknown and need further exploration.

18.2.4 Liver Cancer

There is much evidence that ROS induces protein alterations and DNA injury and thus can act to initiate or promote carcinogenesis (Li et al. 2015; Wang and Feng 2015). For example, the progression of hepatosteatosis to liver cancer by cytoglobin deficiency occurs by the oxidative stress pathway; oxidative stress production by the core protein of hepatitis C virus (HCV) may also partly contribute to the development of hepatocellular carcinoma (Koike 2007). Antioxidants are an important defense system in suppressing tumor initiation and progression and so represent an attractive target in the prevention and treatment of liver cancer. Antioxidant extracts from potatoes inhibits the proliferation of human liver cancer cells (Wang et al. 2011). The antioxidant property of the unicellular green microalgae *Chlorella vulgaris* has antitumor effects against liver cancer, likely by increasing expression of p53, caspase-3, and pro-apoptotic proteins. Our previous studies demonstrated that *Coptidis rhizome* and berberine are potential drugs for the treatment of liver cancer due to their striking hepatoprotective and antioxidant abilities (Tan et al. 2014; Wang et al. 2014). Furthermore, the combination of chemotherapeutic drugs and antioxidants has been reported to lower drug resistance and to sensitize liver cancer cells to chemotherapeutic agents (Xu et al. 2014). Importantly, the effects of antioxidants on liver cancer have primarily been investigated in in vitro studies, and detailed animal experiment and clinical trials are yet to be undertaken.

Table 18.3 The effects of natural products or compounds on liver injury induced by toxins

Models (prevention/treatment)	Materials	Effects	References
Paracetamol-induced liver toxicity in mice (prevention)	Gallic acid; sauchinone; genistein; *Phyllanthus niruri*; *Polyalthia longifolia* leaves	Antioxidation, hepatoprotection	Rasool et al. (2010)
Paracetamol-induced liver damage in rats (prevention)	*Boerhaavia diffusa* leaves; saponarin from *Gypsophila trichotoma*	Antioxidation, hepatoprotection	Olaleye et al. (2010)
Lipopolysaccharide-induced liver injury in rats (prevention)	Carnosic acid	Antioxidation, hepatoprotection	Xiang et al. (2013)
D-Galactosamine-induced liver injury in rats (prevention)	Combination of selenium, ascorbic acid, beta-carotene, and alpha-tocopherol; *Leucasaspera*; swertiamarin from *Enicostemma axillare*	Antioxidation, hepatoprotection	Catal and Bolkent (2008)
Lipopolysaccharide/D-galactosamine-induced liver injury in rats (prevention)	Curcumin; betulinic acid; *Tridax procumbens*	Antioxidation, hepatoprotection	Cerný et al. (2011)
Doxorubicin-induced liver injury in rats (prevention)	N-Acetylcysteine	Antioxidation, hepatoprotection	Kockar et al. (2010)
Cisplatin-induced liver injury in rats (prevention)	Tomato juice	Antioxidation, hepatoprotection	Avci et al. (2008)
Tert-butyl hydroperoxide-induced liver injury in rats (prevention)	Propolis	Antioxidation, hepatoprotection	Wang et al. (2006)
Tamoxifen-induced liver injury in mice (prevention)	Catechin	Antioxidation	Tabassum et al. (2007)
Hepatic steatosis stimulated with tunicamycin (treatment)	Melatonin	↓ ER stress, expression of miR-23a	Kim et al. (2015)
Ethionine-induced liver injury in mice (prevention)	Melatonin	Antioxidation, hepatoprotection	Ferraro and Lopez-Ortega (2008)
CCl_4-induced liver damage in rats (prevention)	*Coptidis rhizome* and berberine	↑ SOD;↓ ALT, AST, Erk1/2	Feng et al. (2011)
CCl_4-induced liver damage in rats (prevention)	Friedelin isolated from *Azima tetracantha* leaves	↑ SOD, CAT, GSH, GPx; ↓ ALT, AST, LDH	Adegbesan and Adenuga (2007)
CCl_4-induced liver damage in rats (treatment)	n-Butanol fraction of *Actinidias deliciosa* roots	↑ GSH; ↓ALT, AST, MDA	Bai et al. (2007)
CCl_4-induced liver damage in rats (prevention)	*Dioclea reflexa* seeds	↑ SOD, CAT; ↓ Transaminases, MDA	Iliemene and Atawodi (2014)

(continued)

Table 18.3 (continued)

Models (prevention/treatment)	Materials	Effects	References
CCl_4-induced liver damage in rats (prevention)	*Pleurotus ostreatus* (oyster mushroom)	↑ GSH, CAT, SOD, GSH- Px; ↓ ALT, AST, ALP, MDA	Jayakumar et al. (2006)
CCl_4-induced liver damage in rats (prevention)	*Cytisus scoparius*	↑ GSH, CAT, SOD, GSH- Px, GST, GRD; ↓ ALT, AST, LDH	Raja et al. (2007)
CCl_4-induced liver damage in rats (prevention)	Ethanol extract of *Phellinus merrillii*	↑ CAT, SOD, GSH-Px; ↓ ALT, AST	Chang et al. (2007)
CCl_4-induced liver damage in rats (prevention)	*Ginkgo biloba*	↑ GSH, SOD, CAT, GSH-Px, GRD, albumin; hepatoprotection	Naik and Panda (2008)
CCl_4-induced liver damage in mice (prevention)	Protein isolate from *Phyllanthus niruri*	↑SOD, CAT; ↓ALT, ALP; lipid peroxidation	Bhattacharjee and Sil (2007)
CCl_4-induced liver damage in mice (prevention)	Kahweol and cafestol *(Coffee)*	↓ALT, AST, cytochrome P450 2E1, lipid peroxidation	Lee et al. (2007)
CCl_4-induced liver damage in rats (prevention)	*Cirsium setidens*	↑ GSH-Px; SOD; hepatoprotection	Lee et al. (2008)
CCl_4-induced liver damage in rats (prevention)	Curcumin and saikosaponin A	↑SOD, GSH; ↓MDA; hepatoprotection	Wu et al. (2008)
CCl_4-induced liver damage in rats (prevention)	Ethanolic extract of *Momordica tuberosa* tubers	Antioxidation, hepatoprotection	Kumar and Deval (2008)
CCl_4-induced liver damage in rats (prevention)	Oregano and rosemary	↓AST, ALT, ALP; antioxidation	Botsoglou et al. (2009)
CCl_4-induced liver damage in rats (prevention)	*Ficus carica* leaves and fruits, *Morus alba* root barks	↑CAT, SOD, GSH; ↓ MDA, AST, ALT, ALP	Singab et al. (2010)
CCl_4-induced liver damage in rats (prevention)	*Podophyllum hexandrum*	↑ GSH, GSH-Px, GRD, SOD, GST; ↓ AST, ALT, LDH	Ganie et al. (2011)
CCl_4-induced liver damage in rats (prevention)	*Ficus religiosa* roots	↑ CAT, GSH-Px, GRD, SOD, GST; ↓ lipid peroxidation; hepatoprotection	Gupta et al. (2011)
CCl_4-induced liver damage in rats (prevention)	Dehydroabietylamine, *Carthamus tinctorious*	↓ AST, ALT, ALP; antioxidation	Paramesha et al. (2011)
CCl_4-induced liver damage in rats (prevention)	Artemetin, *Vitex glabrata*	↑SOD, CAT, GSH-Px; ↓ AST, ALT, ALP, lipid peroxidation, total bilirubin	Sridevi et al. (2012)

Table 18.3 (continued)

Models (prevention/treatment)	Materials	Effects	References
CCl_4-induced liver damage in mice (prevention)	Blueberry anthocyanins	↑ SOD, CAT, GRD, glycogen; ↓ AST, ALT, MDA	(Chen et al. 2012)
CCl_4-induced liver damage in rats (prevention)	*Matricaria chamomilla*	↑SOD, CAT, GSH-Px, GSH; ↓AST, ALT, MDA	Aksoy and Sozbilir (2012)
CCl_4-induced liver damage in mice (prevention)	*Lysimachia clethroides*	↑ SOD; ↓ AST, ALT, MDA	(Wei et al. 2012)
CCl_4-induced liver damage in rats (prevention)	*Garcinia indica* fruit rind	↑ SOD, CAT, GRD, GSH-Px, GSH; ↓ AST, ALT, MDA	Panda and Ashar (2012)
CCl_4-induced liver damage in rats (prevention)	*Agaricus blazei*	↑GSH, GRD; ↓ AST, ALT, MDA	Al-Dbass et al. (2012)
CCl_4-induced liver damage in rats (prevention)	*Nerium oleander* flowers	↑SOD; ↓ AST, ALT, ALP, MDA	Singhal and Das Gupta (2012)
CCl_4-induced liver damage in rats (prevention)	*Hybanthus enneaspermus*	↓ AST, ALT, ALP, total bilirubin; antioxidation	Vuda et al. (2012)
CCl_4-induced liver damage in mice (treatment)	Anthocyanins in black rice bran	↑SOD, GSH-Px; hepatoprotection	Hou et al. (2013)
CCl_4-induced liver damage in rats (prevention)	*Rourea induta*	↑ SOD, CAT, GSH, GSH-Px; ↓ AST, ALT, total bilirubin;	Kalegari et al. (2014)
CCl_4-induced liver damage in rats (prevention)	Proanthocyanidins extracted from grape seeds	↑ SOD, GSH, GSH-Px, CAT; ↓ lipid accumulation, liver injury, DNA damage	Dai et al. (2014)
CCl_4-induced liver damage in mice (prevention)	*Veronica ciliata*	↑ SOD, GSH; ↓ ALT, AST, ALP	Yin et al. (2014)
CCl_4-induced liver damage in rats (prevention)	*Suberea mollis*	↑ SOD, GSH, GSH-Px, CAT; ↓ ALT, AST, ALP, MDA	Abbas et al. (2014)
CCl_4-induced liver damage in rats (prevention)	*Solanum xanthocarpum* leaves	↑ SOD, CAT, GSH, GST; ↓ ALT, AST, ALP, LDH	Jalali Ghassam et al. (2014)
Methidathion-induced liver injury in rats (prevention)	Vitamins C and E	↓ AST, ALT, ALP, MDA;	Sutcu et al. (2006)
Pesticide (chlorpyrifos and cypermethrin)-induced hepatic damage in mice (prevention)	Black tea	↑ SOD, GSH, GSH-Px, CAT, GRD, GST; ↓ AST, ALT, ALP	Khan (2006)
Polychlorinated biphenyl-induced hepatic damage in rats (prevention)	Alpha-tocopherol	Antioxidation	Banudevi et al. (2006)

(continued)

Table 18.3 (continued)

Models (prevention/treatment)	Materials	Effects	References
Aflatoxin-induced hepatic injury in rats (prevention)	*Urtica dioica* seed	↑ SOD, GSH-Px, CAT, GRD, GST; ↓ lipid peroxides, hydroxyl radical, and hydrogen peroxides	Yener et al. (2009)
Thioacetamide-induced hepatic damage in rats (prevention)	Eugenol	↑ COX-2;↓ AST, ALT, ALP, bilirubin, CYP2E1, lipid peroxidation; antioxidation	Yogalakshmi et al. (2010)
Lead-induced liver damage in rats (prevention)	Ginger	↑ SOD, CAT; ↓ MDA,	Khaki and Khaki (2010)
Dimethylnitrosamine-induced hepatic damage in rats (prevention)	Anthocyanins from purple sweet potato	↑ Nrf2, NADPH, GSH, GST; ↓ yclooxygenase-2, MDA	Hwang et al. (2011)
Cadmium-induced hepatic injury in rats (prevention)	Heated garlic juice, ascorbic acid	↑ Nrf2, SOD, CAT; ↓ MDA	Lawal et al. (2011)
Potassium bromate-induced hepatotoxicity of rat (prevention)	*Launaea procumbens*	↑ SOD, CAT, GSH, GSH-Px, GRD, GST	Khan et al. (2012)
Dimethylnitrosamine-induced liver fibrosis in rats (prevention)	*Platycodi radix* root	↑ Nrf2, heme oxygenase-1, NADPH, NQO1, GST; ↓ ALT, AST; anti-fibrotic action	Choi et al. (2013)
As_2O_3-induced hepatotoxicity in cat (prevention)	Resveratrol	↑ GSH; ↓ ROS, MDA	Zhang et al. (2014)
Sodium arsenite-induced liver damage in rats (prevention)	*Emblica officinalis*	Antioxidation	Maiti et al. (2014)
Trichloroacetic acid-induced liver injury in rats (prevention)	Date palm fruit	↑ SOD, CAT, GSH-Px; ↓ MDA	El Arem et al. (2014)

↑ means increase or enhance, ↓ means decrease

18.3 Clinical Trials of Antioxidants in Selected Liver Diseases

18.3.1 Viral Hepatitis

Oxidative stress increases oxidized proteins, impairs nucleic acid and reduces antioxidant defenses in the early stages of viral hepatitis (Ko et al. 2005; Saeki et al. 2006). Concentrations of MDA in chronic hepatitis B (HBV) patients are significantly increased (Tasdelen Fisgin et al. 2012). The level of oxidative stress correlates with the severity of HCV in patients, and there are increases in GSH levels and

reduced activities of antioxidant enzymes such as SOD and GPX (Fujita et al. 2007). Furthermore, oxidative stress is regarded as a risk factor for the development of hepatocellular carcinoma (HCC) in patients with hepatitis. After viral depletion, oxidative stress is normalized, which provides evidence that the virus itself generates oxidative stress (Fujita et al. 2007). In in vitro studies, mitochondrial impairment and oxidative stress induced by hepatitis C virus is calcium dependent and can be inhibited by calcium chelating agents (Wang et al. 2010). However, the absence of a suitable animal model for HCV makes it difficult to undertake preclinical studies of antioxidants for viral hepatitis.

Some clinical trials have evaluated the role of antioxidants therapy in patients with viral hepatitis. Supplementation with Vitamin E in patients with hepatitis C has been assessed in several studies. Although some beneficial effects such as decreased MDA and alanine transaminase (ALT) levels were observed, the clinical significance of these results is uncertain. Subsequently, therapy with a combination of antioxidants was attempted in other studies (Melhem et al. 2005; Gabbay et al. 2007). For example, 50 patients with hepatitis C were treated with several antioxidants including vitamin E. After 20-weeks of treatment, levels of ALT were normalized in 48% of patients and HCV ribonucleic acid (RNA) was negative in 25% of patients, and quality of life was improved in 58% of patients. These results appeared to be promising at first, but in another placebo-controlled randomized study to assess the effect of combination treatment with vitamin C, vitamin E, and selenium for 6 months in 23 hepatitis C patients, the antioxidants failed to improve ALT, HCV RNA, or histology (Singal et al. 2011). Agents such as MitoQ and N-acetylcysteine (NAC) have also been tested in patients with hepatitis C, but with unclear the clinical significance (Farias et al. 2012). The role of agents such as zinc and silymarin in the treatment of hepatitis C patients has also been studied; zinc supplementation improves ALT normalization and could reduce the risk of HCC in patients with zinc deficiency, while silymarin had some positive effects on hepatitis C in several studies, which needs to be confirmed in randomized controlled trials with larger sample sizes (Singal et al. 2011). In summary, although some beneficial outcomes have been reported, there is a lack of studies using randomized, double-blinded clinical trials on the possible merits of antioxidant treatments for patients with hepatitis C.

18.3.2 Alcoholic Hepatitis and Alcoholic Cirrhosis

Markers of oxidative stress and lipid peroxidation are amplified in patients with alcohol-related liver disease. The pro-oxidant and antioxidant levels in patients with chronic alcohol consumption show increases in MDA, and decreases in vitamins E and C were correlated with the severity of the disease in ALD patients (Zima and Kalousova 2005). Significant decreases of GSH levels in liver and blood of patients with ALD were detected; however, the change of SOD and CAT was uncertain, depending on the manner of alcohol intake (Wu and Cederbaum 2009). Enhanced expression of antioxidants enzymes could be a compensatory response to heightened oxidative stress (Li et al. 2015).

Therapy with antioxidant supplements to improve outcomes in patients with alcoholic liver diseases was studied on 20 chronic alcoholic patients; polydatin, a hydroxystilbene derived from the rhizome of *Polygonum cuspidatum* with antioxidant properties, reduced elevated plasma aspartate aminotransferase (AST) and ALT levels in patients while also significantly decreasing lipid peroxidation levels (Pace et al. 2015). The effects of vitamin E supplementation on alcoholic hepatitis and alcoholic cirrhosis were assessed in several randomized double-blind clinical trials. However, vitamin E failed to improve liver function or survival rates in patients. A well-known antioxidant, NAC, has been assessed to treat alcoholic hepatitis in some clinical trials (Singal et al. 2011). In one such study, patients receiving steroids and NAC had lower mortality and lower complication rates when compared to patients treated with steroids alone. The promising therapeutic effects of NAC as an adjuvant treatment on alcoholic hepatitis have yet to be confirmed in a study with a larger population size. Treatment with polyenylphosphatidylcholine improves liver function (as reflected by reduced liver enzymes and serum bilirubin levels) in patients with alcoholic cirrhosis and with HCV infection. Some studies have reported that silymarin has beneficial effects in alcoholic cirrhosis patients. Patients receiving silymarin (420 mg/day) had improved histological results such as inflammatory lesion and necrosis and biochemical findings such as ALT and AST. Another study reported that treatment with silymarin (520 mg/day) improved 4-year survival rates when compared with placebo-treated patients (Singal et al. 2011). But in several other studies, silymarin treatment did not provide any biochemical, histological, or survival benefits. Additionally, a meta-analysis on the use of silymarin in patients with alcoholic cirrhosis concluded that it has no beneficial effect (Singal et al. 2011). This could be due to difficulties in controlling alcohol intake and abstinence rates in many of the studies. In summary, the therapeutic effect of antioxidants on alcoholic hepatitis and alcoholic cirrhosis is unproven.

18.3.3 Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis

Increased serum markers of oxidative stress and lower antioxidant levels occur in NAFLD patients (Singal et al. 2011). Oxidative stress, as a secondary contributor to the progression from NAFLD to NASH, positively correlates with disease progression (Madan et al. 2006). The role of oxidative stress in this disease has not been fully explored with well-designed clinical trials evaluating the effects of antioxidant supplements. Vitamin E has been studied to patients with NAFLD and NASH (Singal et al. 2011). The results of improved oxidative stress without significant clinical efficacy in patients with NASH have limited enthusiasm for this approach, especially since there were no striking improvements in biochemical and histological parameters in these studies (Singal et al. 2011). However, more promising data

for the use of vitamin E was achieved when treatment lasted longer. In a double-blinded placebo-controlled trial with nondiabetic NASH, patients were treated for 96 weeks with either pioglitazone (30 mg/day), vitamin E (800 IU/day), or placebo. Patients treated with vitamin E (but not pioglitazone) had significant improvements in liver biopsy results and improvements in NASH activity scores, while both vitamin E and pioglitazone improved liver enzymes and reduced hepatic steatosis and lobular inflammation (Sanyal et al. 2010).

Other antioxidant agents such as NAC, coenzyme Q10, vitamin D, and betaine have also been evaluated in patients with nonalcoholic liver diseases. An open-label prospective trial of 20 patients treated for 12 months with NASH, NAC (1.2 g/day), and metformin (500 mg/day) improves liver enzymes, insulin resistance, body mass index, steatosis, and fibrosis (de Oliveira et al. 2008). In a randomized double-blinded placebo-controlled trial that enrolled 44 NAFLD patients, waist circumference and AST levels of patients were significantly decreased after 4 weeks of treatment with coQ10 treatment (100 mg/day), suggesting a potential for coQ10 therapy in NAFLD management (Farhangi et al. 2014). In another study of patients with NAFLD receiving vitamin D supplementation, the median of serum 25(OH) D-3 levels significantly increased along with significant decreases in serum MDA, indicating that vitamin D might be effective as an adjunctive therapy to NAFLD patients (Sharifi et al. 2014). Several studies have evaluated the efficacy of betaine in NASH patients. Although some encouraging results were obtained in these studies, it was suggested that the primary underlying mechanism of betaine was as a methyl donor rather than as an antioxidant (Federico et al. 2014).

18.4 Conclusions

This chapter provides an overview of our current understanding of oxidative stress and the actions of antioxidants in liver diseases. On the one hand, the pivotal role of oxidative stress in a broad spectrum of liver diseases has been well documented, and promising data were obtained with antioxidants in animal studies. The underlying mechanisms of action of endogenous and exogenous antioxidants in hepatoprotective effects are unclear. On the other hand, clinical trials studying the potential application of antioxidants alone or in combination therapy in liver diseases are challenging. This might be partly due to the fact that most of the animal studies reflect an early stage of liver disease, where antioxidants might have more satisfactory effects on less advanced liver diseases. In this regard, translational research should be better refined so that the role of antioxidants in more advanced stages of liver diseases can be studied more fully. Variables such as duration of treatment, as well as dose to be used, bioavailability in humans, and mode of administration of antioxidants should be carefully explored. In addition, it would also be important to critically evaluate the study design, clinical endpoints, and patient populations in such clinical trials.

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Chapter 19
Antioxidants in the Prevention and Treatment of Cancer

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Abstract Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly produced in all aerobic organisms, mostly as a consequence of normal cellular aerobic respiration. Many factors outside the body, such as unhealthy diets and behaviours, exposure to environmental pollutants and radiation pollution, also trigger the production of abnormally high concentrations of highly reactive and toxic ROS and NOS in tissues and organs of biological systems. The excessive production of ROS/RNS causes damage to DNA, proteins and lipids and can increase the risk of cancer. Antioxidants maintain redox homeostasis and prevent ROS-/RNS-induced damages that have been associated with cancer development. In the body, antioxidant defence systems include endogenous (enzymatic and non-enzymatic antioxidants) and exogenous antioxidants supplied by plant foods. Plants or parts of plants with medicinal properties are traditionally used in health care and disease prevention and treatment. Plants are considered relatively safe, efficient and inexpensive ways of producing several valuable molecules, including many anticancer drugs. Rational food selection based on therapeutic properties and antioxidant constituents might be a useful strategy for cancer prevention. This chapter summarises recent progress on the production and health benefits of antioxidants derived from food and medicinal plants and their use in cancer prevention and treatment.

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This chapter also outlines most recent anticancer drugs originally derived from medicinal plants and discusses lead structures isolated from natural product with anticancer potential use as adjuvants in conventional anticancer drugs.

Keywords Antioxidants • Healthy diet • Food • Medicinal plants • Cancer prevention and treatment

19.1 Introduction

Noncommunicable diseases (NCDs), mainly cancers, are responsible for about two-thirds of deaths worldwide, mostly in low- and middle-income countries (Ezzati and Riboli 2012). Each year, almost 7 million people die from cancer and close to 11 million new cases are diagnosed, with more than half occurring in the developing world (WHO 2012). The incidence of cancer rises globally because of increased human exposure to environmental pollutants and unhealthy lifestyle factors (Anand et al. 2008, Sankpal et al. 2012, Pacheco et al. 2016). Modern cancer therapies have significantly prolonged the life in many cancer patients but have not succeeded in reducing cancer mortality. Chemotherapy and radiation are toxic to healthy tissues and organs and cause serious side effects. Therefore, patients worldwide use alternative and/or complementary plant therapeutic strategies during cancer treatment (Lee et al. 2014, Wang et al. 2014, Abdallah et al. 2015, Poonthananiwatkul et al. 2015).

The consumption of medicinal plants and herbal remedies is higher than modern medicines largely because of their easy availability, low cost and minimal side effects. In the field of cancer therapy, 49% of the 85 molecules approved between 1940 and the end of 2014 are natural or natural-derived products (Newman and Cragg 2016). Bioactive compounds in plants can be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in human and animals. Plant secondary metabolites have been identified as cancer chemopreventive agents (Kinghorn et al. 2004). Examples of plant-derived anticancer compounds are reserpine (Lupulescu 1983, Abdelfatah and Efferth 2015), quinine (Solary et al. 1992), Taxol (Expósito et al. 2009), curcumin (Park et al. 2013) and aspirin (Alfonso et al. 2014). Many plant-derived anticancer agents including vinblastine; vincristine (isolated from the *Catharanthus roseus* G. Don.); etoposide and teniposide (*Podophyllum peltatum* Linn.); paclitaxel, also named as Taxol (isolated from the bark of *Taxus brevifolia* Nutt.); camptothecin (isolated from the Chinese ornamental tree, *Camptotheca acuminata* Decne); and homoharringtonine (isolated from the Chinese tree *Cephalotaxus harringtonia* var. *drupacea*) are in clinical use against a range of cancers (Prakash et al. 2013).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), including the oxygen free radicals, superoxide radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), nitric oxide ($NO^{\bullet}$) and the highly reactive and toxic, hydroxyl radical ($^{\bullet}OH$) and peroxynitrite ($ONOO^-$), are constantly produced in all aerobic organisms, mainly as a consequence of normal cellular aerobic respiration. Factors

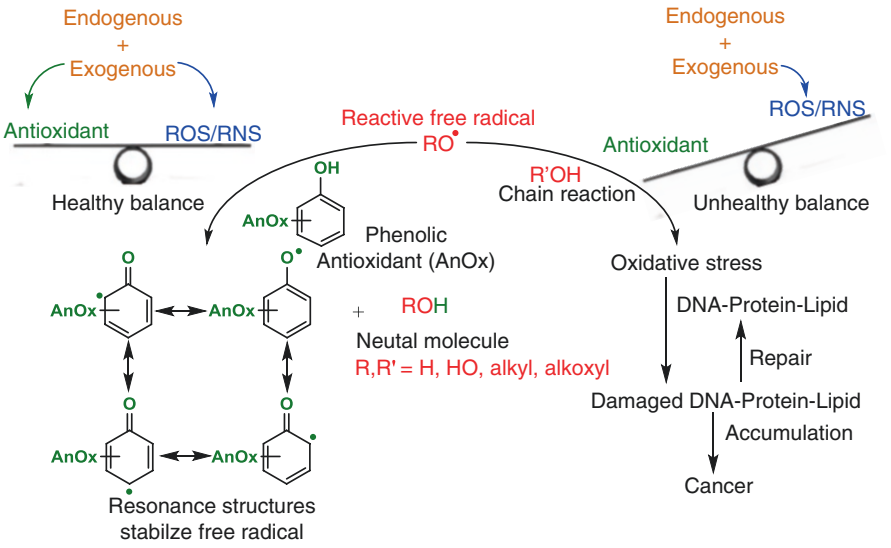


Fig. 19.1 Role of antioxidant-reactive oxygen species (ROS) balance in preventing/promoting cancer

outside the body, such as unhealthy diets (high content in fats, free sugars, salt and low fruit and vegetable intake) and unhealthy behaviours (tobacco smoking, alcohol consumption, physical inactivity), exposure to environmental pollutants (cigarette smoke, nitrogen oxides, sulphur dioxide, particulate matter, polycyclic aromatic hydrocarbons, heavy metals and pesticides) and radiation pollution (ionising and nonionising radiation, ultraviolet radiation and many other radioactive substances) also contribute to the generation of abnormally high concentrations of ROS and NOS (Fig. 19.1) in tissues and organs of biological systems (Sankpal et al. 2012, Poljšak and Fink 2014). The excessive production of ROS/RNS and the resulting oxidative/nitrosative stress may induce damage of cellular macromolecules, including DNA proteins and lipids, and can increase the risk of cancer development (Storz 2005, Ying and Hofseth 2007, Ortega et al. 2010, Reuter et al. 2010). Therefore, modulation of intracellular ROS/RNS levels by antioxidants can be used to target oxidative stress-mediated cancer initiation, promotion and progression.

Antioxidants mainly act as chemical electron quencher and stop or reduce the free-radical chain reaction. The effectiveness of an antioxidant is related to many factors, including activation energy, rate constants, oxidation-reduction potential and solubility properties. The efficiency of an antioxidant increases with decreasing phenolic oxygen-hydrogen bond strength (Shahidi and Naczk 2006). Thus, an antioxidant with a phenolic moiety can readily transfer electron or easily donate hydrogen atom from two phenolic sites to scavenge free radicals; and its radical intermediates are relatively stable due to delocalisation of electrons on the aromatic ring. The stable radical intermediate decreases the formation of new radicals, thus slowing chain free-radical reactions and improves defence efficiency. Rational food selection based on therapeutic properties and antioxidant constituents might be a useful strategy for cancer prevention. Fruit and vegetable antioxidants are crucial for protection against

ROS/RNS molecules that are produced in the human body by the breakdown of nutrients from food and/or after exposure to multiple environmental factors (Balsano and Alisi 2009, Jacob et al. 2012, Aboul-Enein et al. 2013, Poljšak and Fink 2014). Evidence suggests that regular and sustained consumption of fruits and vegetables may reduce the risk of NCDs, including cancer (Potter and Steinmetz 1996, La Vecchia et al. 2001, Riboli and Norat 2003, Benetou et al. 2008, Boffetta et al. 2010, Choi et al. 2015). In this chapter, plant sources of antioxidants and methods to enhance their production will be addressed. The rational use of plant antioxidants designed for cancer prevention will be discussed. This is followed by a brief description of recent anticancer drugs derived from medicinal plants and lead structures with anticancer potential. Finally, the potential interaction of plant medicinal extracts with known anticancer drugs is discussed.

19.2 Plant Antioxidants

Plant primary metabolites are sugars, fatty acids, amino acids and nucleic acids, which are required for plant growth, development and reproduction (Kasote et al. 2015). Plants also produce secondary metabolites with antioxidant properties as an adaptive defence mechanism that provides protection against environmental biotic and abiotic stresses. They are also essential to the normal reproduction, growth and development of plants (Kasote et al. 2015). The plant phenolic compounds include a variety of structures ranging from small molecules (phenolic acids) to complex structures (polyphenols). Plant antioxidant defence systems have also diverse structural features, which include the enzymatic compounds, superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), as well as non-enzymatic water-soluble compounds, such as ascorbic acid, glutathione and flavonoids, and lipid-soluble molecules, such as alpha-tocopherol, beta-carotene and lycopene (Arora et al. 2002, Gill and Tuteja 2010).

19.3 Processing of Plant-Derived Antioxidants

Many reports have investigated the effects of processing, cultivation, packing, temperature and storage conditions on the antioxidant activity in food, fruits and vegetables. Antioxidant phytochemicals present widely in fruits, vegetables, spices, herbs and medicinal plants and belong to various classes of compounds with a wide variety of physical and chemical properties (Carlsen et al. 2010, Fu et al. 2011a, Nahak et al. 2014). A recent study by Castro-Lopez and co-authors evaluated the changes in the overall antioxidant properties of fruit beverages under different storage conditions by measuring the stability of phenolic compounds, ascorbic acid, total carotenoids and total antioxidant activity (Castro-Lopez et al. 2016). These compounds were

stable and in some cases increased during the first 12 days at temperatures between 4 and 8 °C. Carotenoids and ascorbic acid were slightly degraded through the storage period, possibly by oxidation or interaction with other components (Castro-Lopez et al. 2016). The bioactive compounds of the plants have been successfully isolated and identified as our chemical techniques have improved. Extraction of crude drugs depends on the physical nature of the drug and chemical properties of other constituents present in plants (Xu et al. 2017).

Plants cultivated in an organic environment are perceived to be more nutritious, better tasting, and environmentally friendlier compared to food cultivated under conventional conditions. Ren and co-workers studied the relationship between cultivation practices on the production of secondary metabolites in onions (a major source of polyphenols in human diet) grown under conventional, organic and mixed systems (Ren et al. 2017). Phenolics, total flavonoids and antioxidant activities were measured over a 4-year period. Total phenolic and flavonoid contents were generally higher in red onion and were significantly higher in those grown under organic cultivation compared to mixed and conventional treatments (Ren et al. 2017). The antioxidant contents of fruits are strongly influenced by factors such as rootstock (Remorini et al. 2008), climatic conditions and ripening stages (Scordino et al. 2012). Monitoring the nutritional value of fruits during the ripening process is helpful to determine the optimal date for harvesting and to achieve the best quality for both fresh consumption and processing.

Fruit peels are richer in nutritional value than the edible fleshy parts. Fruit peels are usually discarded because they may be indigestible or contaminated by pesticides. However, high concentrations of phenolic compounds are present in fruit peels, making them attractive as source of functional foods and nutraceuticals for health (Varzakas et al. 2016). Peels of peach and nectarine contain at least twice as much phenolics (Tomas-Barberan et al. 2001) and carotenoids and ascorbic acid (Gil et al. 2002) compared to their fleshy parts. Pomegranate peel extracts have more potential as a health supplement rich in polyphenolic antioxidants than the pulp extract (Li et al. 2006). Importantly, the antioxidant activity of fruit flesh tends to increase during ripening, while in the peel this trend is not always the case and can vary in different fruits (Dabbou et al. 2017).

The chemicals used in postharvest technology of horticultural crops must comply with food safety rules and regulations. Fresh fruit and vegetables lose weight due to water loss and respiration after harvest. Ethylene is the ripening promoting agent, leading to crop flesh softening together with increases in the rates of various other ripening processes such as discoloration, weight loss, general senescence and respiration. The antioxidant content is good indicator of the internal cell situation, and fruits with high antioxidant content are considered healthy. Strawberries and their fresh juice show high antioxidant activities that are susceptible to postharvest loss. Methyl jasmonate plays a key role in cell communication including ethylene production, defence responses and increasing the antioxidant capacity of different harvested fruits and horticultural crops. Methyl jasmonate enhances antioxidant activity hence increases the fruit storage period by enhancing the defence systems (Asghari and Hasanlooe 2016).

Plant polyphenols primarily occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups. The cooking process either increases (Dewanto et al. 2002) or decreases (Mazzeoa et al. 2011) polyphenol levels, depending on the type of plants and the cooking procedure (time, pressure, etc.). The polyphenol content in cooked vegetables depends on whether the phenolic moiety exists in a linked or free form. A study of antioxidant activity in cooked carrots (peeled and unpeeled) reported that the antioxidant activity was usually higher in cooked and unpeeled carrots but that it was either unchanged or decreased in peeled carrots (Biezanowska-Kopec et al. 2016).

Mushrooms are frequently used for their unique taste, aroma, nutritional value and medicinal potential, and many species are available worldwide (Pesti 2014). Mushrooms have a high proportion of indigestible fibre and antioxidant constituents. The effects of $NO^{\bullet}$ on the contents of phenolics, flavonoids and antioxidant activity in mushroom have been examined. Postharvest nitric oxide fumigation of mushrooms significantly enhanced antioxidant activity, most likely by inducing the production of secondary metabolites (Dong et al. 2012).

19.4 Antioxidant Activity of Selected Fruits and Vegetables

Grains are important sources of many nutrients including antioxidants, minerals and fibres, and the antioxidant activities of various fractions of grain obtained during milling process have been studied (Miller et al. 2000). The antioxidant activity is highest in bran, whereas refined flour had the lowest activity. The antioxidant activity of dry beans increases with increased redness of the beans; red kidney beans have higher antioxidant activities than grapes, cabbage, blueberries and strawberries (Miller et al. 2000).

A systematic evaluation of antioxidant activity and total phenolic contents of 62 fruits was evaluated using a ferric reducing antioxidant power (FRAP) assay (Fu et al. 2011b), which is based on the ability of antioxidants to reduce ferric (III) ions to ferrous (II) ions (Benzie and Strain 1996). The Trolox equivalent antioxidant capacity (TEAC) assay, based on the ability of antioxidants to scavenge 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid radicals (ABTS), was also used to evaluate the free radical scavenging capacities of 62 fruits. Chinese dates, pomegranates, guavas, sweetsop, persimmons, Chinese wampee (*Clausena lansium*) and plum had the strongest antioxidant activities among the 62 fruits tested, while olives had the strongest free radical scavenging ability.

The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide has been determined (Carlsen et al. 2010), and the antioxidant content of common fruits, vegetables, spices and nuts is summarised in Table 19.1. The data could be used as database to identify and rank diets in terms of antioxidant intake and designing healthy meal plans. The results also suggest that the antioxidant content of plant-based foods is higher than in animal-based foods. Depending on the mean values, one can conclude that plant products such as fruits, nuts, chocolate and berries have from 5 to 33 times higher antioxidant content than

Table 19.1 Antioxidant activities of selected food ingredients (adapted from Carlsen et al. 2010)

Food ingredients	Antioxidant content, mmol/100 g	Food ingredients	Antioxidant content, mmol/100 g
African baobab tree, leaves (dry, crashed)	48.1	Green tea (prepared)	1.5
Allspice (dried ground)	100.4	Maize (white flour)	0.6
Amla (Indian gooseberry, dried)	261.5	Mango (dried)	1.7
Apples	0.4	Millet	1.3
Apples (dried)	3.8	Mint leaves (dried)	116.4
Apple juice	0.27	*Moringa stenopetala* (dried leaves, stem)	11.9
Apricots dried	3.1	*Moringa stenopetala* (fresh leaves, stem)	3.7
Artichoke	3.5	Nutmeg (dried ground)	26.4
Basil dried	19.9	Okra (gumbo) from Mali (dry, flour)	4.2
Bay leaves dried	27.8	Oranges	0.9
Bilberries dried	48.3	Orange juice	0.64
Beans	0.8	Oregano (dried ground)	63.2
Black tea prepared	1.0	Papaya	0.6
Black olives	1.7	Peanuts roasted with pellicle	2.0
Blueberry jam	3.5	Pecans with pellicle	8.5
Broccoli (cooked)	0.5	Pistachios	1.7
Buckwheat white flour	1.4	Plums, dried	3.2
Buckwheat whole meal flour	2.0	Pomegranate	1.8
Chestnuts with pellicle	4.7	Pomegranate juice	2.1
Chilli red and green	2.4	Prunes	2.4
Cinnamon sticks and whole bark	26.5	Prune juice	1.0
Cinnamon dried ground	77.0	Red wine	2.5
Clove dried, whole and ground	277.3	Rosemary (dried ground)	44.8
Cocoa with milk	0.37	Saffron (dried ground)	44.5
Coffee prepared	2.5	Saffron (dried stigma)	17.4
Cranberry juice	0.92	Sage (dried ground)	44.3
Crisp bread (brown)	1.1	Strawberries	2.1
Curly kale	2.8	Sunflower seeds	6.4
Dates (dried)	1.7	Thyme (dried ground)	56.3
Dill (dried ground)	20.2	Tomato juice	0.48
Dog rose (wild, dried)	78.1	Walnuts with pellicle	21.9
Espresso (prepared)	14.2	Wheat bread (toasted)	0.6
Estragon (dried)	43.8	Whole wheat bread (toasted)	1.0
Fruit from the African baobab tree	10.8	Walnuts with pellicle	21.9
Ginger (dried)	20.3	Zereshk (red sour berries)	27.3
Grape juice	1.2		

the means of meat products. Spices and herbs, although contributing little in terms of weight to a meal, make a large contribution to antioxidant intake. Breakfast cereals, chocolate-containing foods, coffee and tea are important sources of dietary antioxidants. Of note is that the antioxidant content in human breast milk is comparable to pomegranate juice, strawberries and coffee and higher than commercially available infant formula milk. Remarkably, dried fruits are higher in antioxidants than their corresponding fresh fruits and fruit juice. Red cabbage, red beans and red grapes are relatively high in antioxidants compared to their green counterparts. There is a striking difference between red and green cabbage, with the purple cabbage pigment contributing a high level of antioxidant activity. Many popular drinks tend to have relatively moderate levels of antioxidants. In general, spices have the highest antioxidant levels followed by nuts, fruits and vegetables. The antioxidant activity of melons is much lower than other fruits.

In summary, fruit and vegetable antioxidants exhibit health beneficial effects at least in part through prevention of ROS-induced DNA, protein and lipid oxidative damages. Fruit and vegetable antioxidant contents are highly dependent on cultivation, processing, packing, storage conditions and temperature. To enhance or maintain antioxidant content, storage temperature is kept between 4 and 8 °C for 2 weeks, as higher temperatures and or longer times lowering antioxidant content. The entire tissue of fruits and vegetables is rich in phenolic compounds, and in most cases the waste by-products, such as peels, can present higher content of antioxidants. Cultivating fruits and vegetable in organic media improves their antioxidant activity, and postharvest use of chemicals such as methyl jasmonate and nitric oxide significantly enhances antioxidant activities particularly in strawberries and mushrooms, respectively.

19.5 Nutrition and Cancer Prevention

Philosophers, scholars and scientists have for some time tried to develop a relationship between food and therapy, as food and medicinal plants were the only sources for treating different diseases at that time. As chemical techniques became more advanced, treatment of diseases became more reliant on specific molecules prepared under controlled conditions. Although single molecule treatment strategies were successful in treating many diseases, they were expensive and unaffordable in many societies. However, many therapeutic agents were unable to treat NCDs like cancer, leading scientists to re-examine the use of food and medicinal plants as a source of disease prevention and therapy (Back et al. 1995).

19.5.1 Antioxidants for Cancer Prevention

Cancer is a chronic disease with many causes, but unhealthy diets and lifestyle behaviours, as well as exposure to a wide variety of environmental pollutants, may increase the risk at least in part through ROS and RNS generation which induced oxidative stress and lead to cancer initiation and development (Waris and Ahsan

2006, Preedy 2014). Therefore, a fine balance between the levels of ROS/RNS and antioxidants within the cell is crucial for normal physiological function (Fig. 19.1). Any impairments of the redox systems can result in a redox imbalance, consequently promoting damage to key cellular structures including DNA, proteins and lipids, which play a pivotal role in the development of cancer (Fig. 19.1). Since DNA damage from micronutrient deficiencies is likely to be a major cause of cancer (Ames 2001) and ROS appear to play an active role in the development of cancer (Waris and Ahsan 2006), there has been much investigation of the role of nutritional antioxidant intake in the prevention of cancer (Mut-Salud et al. 2016). Some studies have focused on the effects of increasing the dietary intake of vitamins, carotenoids and/or minerals on prostate (Santillo and Lowe 2006), rectal (Hu et al. 2007), gastric (Lazarević et al. 2011), pancreatic (Bravi et al. 2011) and breast (Saquib et al. 2011) cancer.

The use of antioxidants during cancer treatment could interfere with the actions of prescribed anticancer drugs to decrease drug efficacy and prevent cancer cells from undergoing apoptosis. Data from randomised clinical trials show benefit of supplementation with beta-carotene, vitamin C and vitamin E, selenium and zinc alone or in combination on cancer prevention, selenium and zinc alone or in combination with other antioxidant supplements (Hajhashemi et al. 2010, Greenlee et al. 2012, Huang et al. 2016). Antioxidant supplementation, particularly with beta-carotene and vitamin E, does not reduce primary cancer incidence or cancer mortality (Jiang et al. 2010, Chen and Alpert 2016). Selenium supplementation may reduce cancer incidence and cancer mortality in men, but not in women, but clearly more studies are needed to confirm the cancer preventive effects of selenium (Bardia et al. 2008). The standard guideline for patients being treated for cancer is that supplements should not be taken before, during and after the treatment, whereas antioxidants from dietary sources are not restricted. The general conclusion is that while antioxidant supplementation may not prevent cancer formation, healthy diets likely do with organically grown food having high antioxidant contents (Chhabra et al. 2013). Plants grown free of pesticides will maintain high levels of antioxidants. Organic plants are rich sources of in vitamins, minerals and fibres, which facilitate digestion, absorption, distribution and elimination of antioxidants (Volpe et al. 2015, Dietz et al. 2016).

19.5.2 Healthy Nutrition for Cancer Prevention

Many would agree that humans evolved as omnivores, and healthy nutrition usually includes foods derived from plant and animal sources. Now, a healthy diet is seen as one designed to reduce excess body fat and limit the intake of drinks with high sugar, but there are as many healthy dietary patterns as there are concepts of what constitutes a healthy nutrition (Kushi et al. 2012). The consumption of plant foods with a variety of nutrients is more beneficial over single constituent, because interactions between food phytochemicals are important for cancer prevention (Liu 2004).

Colorectal cancer is the second most diagnosed cancer in females and the third most commonly diagnosed cancer in males worldwide (Torre et al. 2015). It is thought that 50–80% of colorectal cancers may be due to environmental factors, mainly unhealthy dietary habits (Karagianni and Triantafillidis 2009). Red meat, processed meat, cheese, alcoholic drinks as well as foods containing iron, animal fats and sugars can lead to colorectal cancer (Zaharek-Girgasky et al. 2015). Recommendations on specific antioxidant supplements, while important, reveal no benefit of antioxidant use among stage-II colorectal cancer survivors (Tsinovoi et al. 2017). Foods containing dietary fibre, as well as garlic, milk and calcium, may protect against colorectal cancer. Similarly, there is limited evidence suggesting that non-starchy vegetables, fruits, foods containing folate, fish, and foods containing vitamin D and selenium may protect against colorectal cancer. Fibre derived from fruits, vegetables and grains is an important component of nutrients. Despite limited data, many experts recommend diets with high fibre content, although any unfavourable effects are not known (Chiba et al. 2015). Dietary fibre is thought to protect against colorectal cancer at least in part by increasing production of short-chain fatty acids, stool mass, and by decreasing colonic transit time, change in colonic pH and modulation of bile acid metabolism (Sehdev and O'Neil 2015).

Chemotherapy significantly improves clinical outcomes in cancer patients but can also result in toxic effects. In oesophageal cancer, treatment with 5-fluorouracil (5-FU)/cisplatin plus docetaxel increases response rates but is also associated with an increased toxicity (Tanaka et al. 2016b). Gastrointestinal toxicity caused by chemotherapy adversely affects nutritional status by decreasing food intake, which can lead to delay or discontinuation of chemotherapy. Oral mucositis, one of the most common gastrointestinal toxicities, results in increased pain, difficulty in swallowing, nutritional compromise and increased risk of infection. A diet containing glutamine induces oral mucositis in oesophageal cancer patients receiving chemotherapy; whereas a diet containing glutamine was administered before starting chemotherapy, glutamine and other minerals helped to maintain nutritional status and reduce the incidence of oral mucositis in oesophageal cancer (Tanaka et al. 2016a).

Dietary polyphenols function as chemopreventive agents and can inhibit conversion of normal cells into cancer cells. The methanol extract of dried pomegranate peels contains 44% phenolic compounds, with the presence of gallic acid and catechin as major components (Murthy et al. 2004). Pomegranate peel extract tends to inhibit the development of colonic premalignant lesions in an azoxymethane-induced colorectal carcinogenesis in rat (Waly et al. 2012). Pomegranate-derived components inhibit DNA damage, which is a key event involved in the initiation phase of cancer development (Turrini et al. 2015). Pomegranate peel extract inhibits cell proliferation of the human immortalised myelogenous leukaemia cells, K562 cells, mainly by cell cycle arrest and apoptosis induction (Asmaa et al. 2015). Powder of dried pomegranate peel contains 30% polyphenols (Folin-Ciocalteu method, equivalent gallic acid), and the concentrations of punicalagin and ellagic acid are 8% and 5%, respectively (Al-Gubory et al. 2016). The antioxidant activity of pomegranate fruit resides in its ability to reduce the production of ROS and RNS and to increase the enzymatic activity of SOD, GPX and CAT (Turrini et al. 2015).

Healthy mice fed with pomegranate peel in diet exhibit lower plasma MDA concentrations, reduced content of MDA in the small intestine and liver and higher levels of Cu/Zn-SOD (SOD1) and GPX activities in the small intestine compared to mice fed the control diet (Al-Gubory et al. 2016). There is substantial evidence that pomegranate-derived products are promising chemopreventive/chemotherapeutic agents, as they exert anti-inflammatory, anti-proliferative and anti-tumorigenic effects by modulating multiple signalling pathways (Sharma et al. 2017).

Curcumin (a naturally occurring compound found in the turmeric spice), quercetin (the most abundant dietary flavonoids found in many fruits and vegetables) and green tea (epigallocatechin-3-gallate) display anti-carcinogenic properties alone or in combination. There is substantial data indicating that curcumin has high antioxidant and ROS-/RNS-scavenging properties (Sreejayan and Rao 1997, Das and Das 2002, Kim et al. 2003, Sumanont et al. 2004, Ak and Gülçin 2008, Barzegar and Moosavi-Movahedi 2011). Importantly, curcumin inhibits carcinogenesis in multiple organs in various animal models (Das and Vinayak 2015). Irrespective of the route of administration, curcumin has low bioavailability, though metabolites of curcumin remain for longer periods in different tissues. Activation of the antioxidant defence systems contributes to cancer prevention (Das and Vinayak 2012). The effects of curcumin in cancer prevention may occur via modulation of stress-activated genes by inducing phase-II antioxidant enzymes, activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signalling and restoration of the tumour suppressor p53 gene (Das and Vinayak 2014).

Green tea is the most widely consumed beverage after water (Yang et al. 2009). Studies in animal models of carcinogenesis show that green tea inhibits tumorigenesis during the initiation, promotion and progression stages. Green tea polyphenols have dual effects and act as antioxidants and/or pro-oxidants to exert preventive effects against cancer (Forester and Lambert 2011). The dual effects of green tea can either induce oxidative stress (leading to ROS-mediated cancer cell death) or scavenge ROS under conditions of high oxidative stress (preventing cellular damage).

Curcumin and green tea catechin alone or in combination can inhibit the proliferation of the human colon adenocarcinoma HCT 15 and HCT 116 cells and the human larynx carcinoma Hep G-2 cells through induction of apoptosis (Manikandan et al. 2012). Combined treatment with curcumin and quercetin induces apoptosis through the mitochondrial pathway and inhibits proliferation of human gastric cancer MGC-803 cell (Zhang et al. 2015b). Therefore, combining chemically similar or different polyphenols can be used as an efficient anticancer treatment (Niedzwiecki et al. 2016).

Soybeans are rich in polyphenols, proteins and fibres. Isoflavones are a subclass of flavonoids found mainly in soybeans and act as antioxidants to inhibit oxidative stress and regulate cellular signalling in cancer. Soy isoflavones have an important role in reducing the incidence of hormone-sensitive cancers, or hormone-dependent cancers, such as breast and prostate cancers (Amaral et al. 2017, Rienks et al. 2017). Isoflavone-based antioxidants from soybeans target multiple signalling pathways, particularly those that are related to the homeostatic control of cell cycle and

apoptosis (Sarkar and Li 2002, 2003), leading to inhibition of cancer development and progression. Isoflavones belong to the phytoestrogen group and have some structural similarity with oestradiol, causing isoflavones to also exert weak oestrogenic activity. Since oestrogen plays an important role in breast cancer development and survival, many questions remain open about the risks and benefits of diets high in soy (Fox et al. 2008). Clearly more clinical trials are needed to evaluate the effects of isoflavone, either alone or in combination with conventional drugs, for the prevention and treatment of cancer.

19.5.3 Rational Diet Design for Cancer Prevention

Many diets are based on regional and cultural influences, and the relation of the nutrient contents and benefits of various diets on health and prevention of disease has been studied by many groups. A Mediterranean diet is considered to be one of the healthiest diets and is based on large amount of fresh fruits, vegetables, whole grains, olive oil and herbs (Potentas et al. 2015), making it rich in vitamins, polyphenols, minerals, carotenoids, lycopenes, resveratrol and other antioxidants that may have anticancer properties (John and Anderson 2014). The Mediterranean diet can be a preventative measure in breast cancer, especially in postmenopausal women (Fung et al. 2005). There is an inverse association between consumption of a Mediterranean diet supplemented with extra virgin olive oil and breast cancer incidence (Mourouti and Panagiotakos 2016, Toledo et al. 2015). More specifically, a Mediterranean diet supplemented with extra virgin oil is associated with a 62% lower risk of malignant breast cancer (John and Anderson 2014).

Diet and body weight are related and may contribute to cancer risk (Nomura et al. 2016, Kohler et al. 2016). A healthy weight is usually determined by using weight and height to calculate body mass index (Ferrer et al. 2016). Consuming a healthy diet helps in maintaining a healthy weight and provides nutrients that may assist in preventing cancer (Bail et al. 2016). A diet high in sugar and fat promotes obesity, which may indirectly increase cancer risk. Fat intake raises the levels of prolactin and oestrogen, which can facilitate the development of breast cancer (Potentas et al. 2015, Toledo et al. 2015). Managing cancer prevention doesn't mean that sugar- and fat-containing foods must be completely removed from the diet. In fact, many foods contain nutrients that are essential to good health; however, their intake must be carefully controlled and physical activity should be included as part of a healthy lifestyle behaviour (Rennie et al. 2015).

Data on the health benefits of antioxidant supplements are ambiguous and difficult to generalise (Hong et al. 2015); hence institutional recommendations on antioxidant supplements tend to be more conservative (Tsinovoi et al. 2017). Health-care experts are careful to recommend antioxidant therapies unless they are able to show benefits and understanding of their toxicity, side effects and treatment responses.

Healthy diets that maintain a healthy weight are primarily plant based; low in red and processed meats; low in simple sugars, refined carbohydrates and saturated fatty acids; and limited alcohol consumption. Common features for healthy diet include:

1. High consumption of fruits and vegetables, spices, nuts and whole grain
2. Moderate consumption of seafood, milk and dairy products
3. Low consumption of meat, soft drinks, alcohol and processed food
4. Olive oil as the main source of fat

Fruit and vegetables fall into five different colour categories: red, purple/blue, orange, green and white/brown. Each colour carries its own set of unique disease-fighting chemicals. A diverse combination of leaf shape and fruit and vegetable colours with the above options (fresh and organic quality) will provide the greatest synergic effect in cancer prevention.

19.6 Anticancer Agents Derived from Medicinal Plants

The World Health Organization (WHO) estimated that the plant-derived drug trade was worth US$100 billion in 2007 and is expected to reach US$5 trillion by 2050 (Greenwell and Rahman 2015). Advances in synthesis of pharmaceuticals can generate new drugs from medicinal plants that are more effective in cancer treatment. However, many pharmaceutical companies stopped investing in natural product research and focus primarily on rational drug design using high-throughput screening and combinatorial and computational chemistry (Wermuth and Aldous 2015). Drug design is based on single small molecule with low molecular weight, chiral centres and hydrogen as donor and acceptor atoms.

The drug design concept supports Lipinski's five rules (RO5) and produced many useful drugs. This is based on analysis of the physicochemical properties of more than 2000 drugs and candidate drugs in clinical trials. RO5 predicts that poor absorption or permeation is more likely when there are more than five hydrogen-bond donors and ten hydrogen-bond acceptors, the molecular weight is greater than 500 Da and the octanol-water partition coefficient log P is not greater than 5 (Lipinski et al. 2001). The origin of the rule's name is based on the fact that all numbers are multiples of five. Unfortunately such approaches are not equally successful in the design of more effective and less toxic anticancer drug. Cancer is a complex disease requiring treatments with either complex molecules with many chiral centres that can act specifically on cancer cells or combinational therapy where two or more drugs are likely more effective treatment (Price et al. 2008). Several cancer chemotherapeutics originate from medicinal plants, largely because they provide structurally complicated molecules that are difficult to synthesise chemically and/or be synthesised in significant quantities (Seyfried et al. 2016).

Many drug-derived plants have been approved, with many more leads being generated (Wermuth and Aldous 2015).

19.6.1 Approved Anticancer Drugs Derived from Medicinal Plants

Plants are considered relatively safe, efficient and inexpensive sources for producing several drugs, as evidenced by the fact that 70–95% of the population in developing countries using traditional medicines (Fridlender et al. 2015). Herbal medicine is used as a complementary treatment with conventional drugs (Robinson and Zhang 2011). Studies of medicinal plants as potential sources of anticancer agents were started in the 1940s and about 175 molecules approved for cancer treatment since then; 75% of approved molecules are derived from a non-synthetic approach of which about 49% is produced directly or derived from natural products (Newman and Cragg 2012, Cragg and Newmann 2013). Moreover, between 2012 and 2014, 18 natural product-based anticancer drugs were approved: 3 as natural products, 3 as natural product derivatives and 12 as a natural product pharmacophore or mimic of natural product (Newman and Cragg 2016) (Table 19.2). An important issue related to plant-derived drugs is around copyright claims, and one solution to resolve this is the use of semi-synthetic or synthetic analogues. Another issue is that some anticancer drugs derived from medicinal plants do not comply with Lipinski's RO5 (Ganesan 2008) as they are highly complex molecules characterised by high molecular weights and rich of stereogenic centres; thus some physicochemical properties of active natural products such as lipophilicity and solubility are not always well represented in synthetic analogues. Therefore, some plant-derived drugs require manipulation of the chemical structure by generating simple derivatives to improve bioavailability while maintaining or enhancing anticancer activity.

19.6.2 Potential Anticancer Drugs Derived from Medicinal Plants

Medicinal plants are rich sources of lead compounds, which often require further fine-tuning of their physicochemical properties (Cragg and Newmann 2013). Identification of lead compounds is the starting point for further development in drug design; reducing their poor solubility, bioavailability, toxicity and moderate activity are key features for further manipulation and optimisation. Medicinal chemists at pharmaceutical companies usually initiate intensive programme of structure-property relationship to improve lead compounds with anticancer activity, as well as their bioavailability and toxicity. Lead compound is a new pharmacophore with the potential to generate molecules with unique properties and reduced cross-resistance

Table 19.2 List of selected approved drugs derived from natural sources (Newman and Cragg 2016)

Generic name	Trade name	Mechanism	Approval year	Cancer type
Blinatumomab	Blincyto	Binds to CD19, CD3 expressed on the surface of B-and T cells	2014	Blood cancer Leukaemia
Nivolumab	Opdivo	Blocks a negative regulator of T-cell activation and response	2014	Metastatic melanoma
Obinutuzumab	Gazyva	Targets the CD20 antigen expressed on the surface of pre B- and mature B-lymphocytes	2013	Chronic lymphocytic leukaemia
Ramucirumab	Cyramza	Binds VEGF receptor 2 and blocks binding of VEGFR ligands, VEGF-A, VEGF-C and VEGF-D	2014	Gastric cancer
Paclitaxel nanoparticles	Taxol	Microtubule inhibitor that promotes the assembly of microtubules from tubulin dimers and stabilises microtubules by preventing depolymerisation	2014	Lung cancer
Trastuzumab emtansine	Kadcyla	Upon binding to subdomain IV of the HER2 receptor	2013	Breast cancer
Apatinib mesylate	Aitan	Tyrosine kinase inhibitor that selectively inhibits the VEGF receptor 2 (VEGFR2, also known as KDR)	2014	Gastric carcinoma, metastatic breast cancer, advanced hepatocellular carcinoma
Ceritinib	Zykadia	Inhibitor of anaplastic lymphoma kinase	2014	Non-small-cell lung cancer
Afatinib	Gilotrif	Covalently binds to the kinase domains of EGFR, HER2 and HER4 (ErbB4) and irreversibly inhibits tyrosine kinase autophosphorylation	2013	Non-small-cell lung cancer
Belinostat	Beleodaq	Histone deacetylase inhibitor	2014	Non-Hodgkin's T-cell lymphoma
Dabrafenib mesylate	Tafinlar	Inhibitor of some mutated forms of BRAF kinases, as well as wild-type BRAF and CRAF kinases	2013	Melanoma Metastatic melanoma
Ibrutinib	Imbruvica	Inhibitor of Bruton's tyrosine kinase (Btk)	2014	Leukaemia Chronic lymphocytic leukaemia

(continued)

Table 19.2 (continued)

Generic name	Trade name	Mechanism	Approval year	Cancer type
Idelalisib	Zydelig	Inhibitor of phosphoinositide-3 kinase (PI3K) delta	2014	Chronic lymphocytic leukaemia, follicular lymphoma
Regorafenib	Stivarga	Targeting angiogenic, stromal and oncogenic receptor tyrosine kinases (TK)	2013	Gastric cancer
Trametinib	Mekinist	Reversible inhibitor of mitogen-activated extracellular signal regulated kinase 1 (MEK1) and MEK2	2013	Melanoma Metastatic melanoma

potential (Cragg and Newmann 2013, Sarker and Nahar 2012, Sharma and Gupta 2015). Selected lead structures with anticancer property isolated in 2016 are presented in Table 19.3.

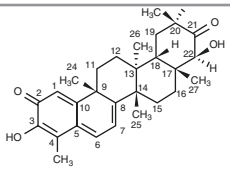
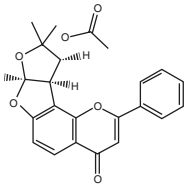
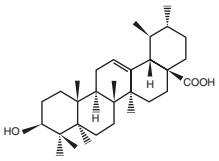
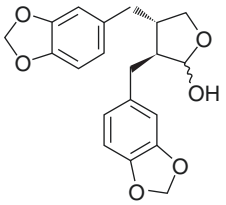
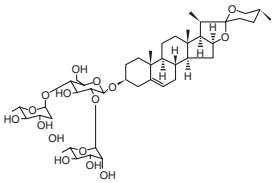
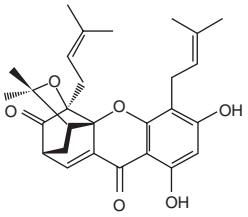
Tingenin b (22β-hydroxytingenone) was obtained from *Maytenus spinosa* (an endemic shrub native to South America) and has promising anticancer property against breast cancer as it induces apoptosis in the breast stem cells, MCF-7 (Cevatemre et al. 2016). (−)-Pseudosemiglabrin was isolated from the aerial parts of *Tephrosia apollinea* (dwarf shrub widely distributed in Africa) and its structural and stereochemical features described by X-ray (Ahmed Hassan et al. 2014). The cytotoxicity of (−)-pseudosemiglabrin was evaluated in nine cancer cell lines where it had dose-dependent anti-proliferative effects on most of the cancer cell lines, with significant inhibitory effect on the proliferation of leukaemia, prostate and breast cancer cell lines but with no toxicity in normal human fibroblasts (Ahmed Hassan et al. 2014).

Ursolic acid, a triterpenoid, was isolated from the ethyl acetate extract of *Betula utilis* (a birch tree native to the Himalayas) bark along with other triterpenoids that were characterised by spectroscopic methods (Mishra et al. 2016). Ursolic acid was tested for in vitro cytotoxic activity against six different cancer cell lines and was selective for breast cancer cells over normal breast epithelial cells (MCF 10A). Cancer cell selectivity is mainly due to the activation of the extrinsic apoptosis pathway via up-regulation of death receptors 4 and 5 (DR4, DR5).

(−)-Cubebin isolated from the defatted acetone extract of *Piper cubeba* seeds can be converted to four different derivatives to get insight on its structure-activity relationship. All compounds were characterised by nuclear magnetic resonance and mass spectrometry and tested for anticancer property against six human cancer cell lines (A549, K562, SiHa, KB, HCT116 and HT29), with the natural lignan (−)-cubebin having high effect against A549, K562 and KB cell lines but being less effective against other cell lines (Rajalekshmi et al. 2016).

Dioscin, a natural steroid saponin, isolated from the root extract of *Dioscorea villosa* (wild yams, a tuberous vine that is native to China and North America), was identified and tested for purity with chromatographic (TLC, LC-ELSD) and spectral

Table 19.3 List of recent leads isolated from natural product with potential anticancer activity

Plant	Name	Structure	Anticancer activities IC50 (48 h)	Target tissues in vitro
Maytenus sp.	Tingenin B		2.38 μM	MCF-7
Tephrosia apollinea	(−)-Pseudosemiglabrin		18.2 μM	MCF-7
Betula utilis	Ursolic acid		2.5–5 μM	MCF-7
Piper cubeba	(−)-Cubebin		8.16–8.66 μM	A549, K562KB
Dioscorea villosa	Dioscin		4.4 μM	HeLa
Garcinia hanburyi	Forbesione		8.0 μM	Ham-1

(IR, 1D- and 2D-NMR, ESI-HRMS) analysis (Aumsuwan et al. 2016). Using human breast cancer cell line MCF-7 (oestrogen receptor positive) and MDA-MB-231 (oestrogen receptor negative) under a hormone-free environment, dioscin reduced cell viability of both cell lines in a concentration- and time-dependent manner and did alter the expression of several genes (especially in MDA-MB-231 cell line) that

encode for proteins involved in the regulation of cellular function such as cell growth, proliferation and migration. The findings support the potential therapeutic ability of dioscin as an anticancer agent in invasive breast cancer. Forbesione, a caged xanthone isolated from *Garcinia hanburyi* (small- to medium-sized Asian tree), has antitumour effect on cholangiocarcinoma in in vitro and in vivo settings. Forbesione activates multiple mechanisms, including induction of S-phase cell cycle arrest and stimulation of the death receptor pathway (Bouery et al. 2016). Forbesione alters the expression of genes and proteins related to cell cycle and apoptosis regulation, and hamsters treated with forbesione for 4 weeks showed no toxic side effect, suggesting that forbesione represents a promising anticancer drug candidate (Bouery et al. 2016) that deserves further investigation.

19.6.3 Combination of Medicinal Plant Extracts with Anticancer Drugs

Medicinal plants are often used together with traditional cancer therapy to improve survival rate and quality of life. Some medicinal plant extracts used by cancer patients either improve anticancer drug efficiency or reduce the toxicity induced by the chemotherapy (Guerriero et al. 2017, Zhang et al. 2016). Earlier studies reported that a plant extract derived from *Caesalpinia spinosa* (Molina, a small leguminous tree or thorny shrub native to south America) reduced spleen metastasis in mice that were transplanted with the murine mammary tumour cell line, 4T1 (Urueña et al. 2013). More recently, Molina was further evaluated in highly resistant human cancer cell lines with or without multidrug resistance phenotypes, such as MES-SA/Dx5, K562, 4T1 and TS/A cell lines (Sandoval et al. 2016). The synergistic effect of Molina extracts and doxorubicin was evaluated in vitro and in vivo using mice transplanted with TS/A cells. The ethanol extract of Molina was cytotoxic to cells regardless of their resistance phenotype but had a synergistic effect with doxorubicin in MES-SA Dx5 Pgp + cells while also increasing survival in TS/A (mouse mammary carcinoma) cell lines. These data suggest that treatment with Molina could be used as an adjuvant with conventional chemotherapy to treat multidrug-resistant tumours.

The bark of *Cinnamomum cassia* (cinnamon, a tree widely cultivated in southern and eastern Asia) is frequently used as spice. It has high antioxidant content (Lin et al. 2003) and potential anticancer (Lee et al. 2004) activities. Cis-diamminedichloroplatinum (CDDP) is one of the most important chemotherapeutic agents for cancer treatment (Strumberg et al. 2002). The aqueous extract of cinnamon exerts protective effects against the toxicity induced by CDDP in the cancer cells, MCF-7 and HepG2, by preventing the activation of various cellular mechanisms mediating apoptotic cell death, without compromising the anticancer efficiency of CDDP (Elkady and Ramadan 2016). *Piper nigrum* (black pepper) is a medicinal plant that possesses a potent cytotoxic effect on breast cancer cell lines as investigated in studies of N-nitrosomethylurea (NMU)-induced mammary tumori-

genesis in rats (Sriwiriyajan et al. 2016). The dichloromethane extract of *Piper nigrum* had potent cytotoxic effects against MCF-7 breast cancer cells compared to colorectal, lung and neuroblastoma cells. Moreover, the extract inhibited mammary tumorigenesis in rats without significant effect on the liver and bone marrow. The bioactive ingredients *in Piper nigrum* need to be isolated and identified for further studies. The combinational effects of natural products and known anticancer drugs have recently been reviewed (Cai et al. 2016, Clark and Lee 2016, Farzaei et al. 2016).

19.6.4 Potential Anticancer Extracts from Medicinal Plants

Phytochemical compounds extracted from *Maytenus spinosa* were active against six solid tumour cell lines at micromolar concentrations (de Almeida et al. 2010). Compounds from *Betula utilis* have antioxidant, anti-inflammatory and anticancer properties (Singh et al. 2012). In an attempt to investigate the influence of extraction solvents on the anticancer properties of medicinal plants, 35 extracts were screened for cytotoxic activities against three different cancer cell lines (B16F10, MCF-7 and HeLa) (Alzeer et al. 2014). Acetone consistently gave lower extraction yields but was more cytotoxic, whereas other solvent systems had much higher extraction yields with lower cytotoxicity. The acetone extract of *Salvia officinalis* L. (sage) has potent anticancer property (IC_{50} = 14–36 μg/ml) in the three cell lines. Interestingly, coconut water is a potential alternative to classical organic solvents as it consistently provides the highest extraction yields and increased the sensitivity of *S. officinalis* L in the human breast cancer cell line MCF-7.

Breast cancer is the most common and prevalent cancer and one of the leading causes of death among women worldwide. The aqueous extract of *Urtica dioica* (a widespread and perennial herbaceous plant in Europe and North America) showed antioxidant effects, induced apoptosis as demonstrated by DNA fragmentation and inhibited proliferation of the human breast cancer cell line, MCF-7 (Fattahi et al. 2013). Methanolic crude extract of *Piper cubeba* seeds has a cytotoxic activity against the breast cancer cell lines, MCF-7 and MDA-MB-468, with low cytotoxicity against normal fibroblast L929 cells (Graidist et al. 2015). *Piper nigrum* extract upregulated p53 and downregulated oestrogen receptor, E-cadherin, matrix metalloproteinases 9 and 2 and vascular endothelial growth factor levels in breast cancer rats (NMU)-induced mammary tumorigenesis in rats, suggesting that *Piper nigrum* can enhance breast cancer cell response to phytochemicals, then induce cell cycle arrest and inhibit cancer cell proliferation (Deng et al. 2016).

Colorectal cancer is the third most common cancer in the world (Haggar and Boushey 2009). Hydroalcoholic extract of *Urtica dioica* inhibited proliferation of gastric and colorectal cancer cells at least in part by inducing apoptosis, while it had no toxic effect on normal cells (Ghasemi et al. 2016). Ethanol extract of *Sorbus rufopilosa* (small ornamental trees used to produce jams and wine and have high

antioxidant content) induced G2/M arrest and apoptosis and inhibited proliferation of human colon adenocarcinoma HT29 cells (Oh et al. 2016). Apoptosis in HT29 cells was associated with p53 up-regulation through both extrinsic and intrinsic pathways. The extract showed higher sensitivity in HT29 cells over HepG2 and A549 cells (Oh et al. 2016).

Actinidia arguta (hardy kiwi fruit or baby kiwi fruit) is known for its good taste and as a healthy food. To enrich its antioxidant and health potential, 1-methylcyclopropene was used during postharvest processing to delay kiwi fruit ripening and softening (Lim et al. 2016). The methanol/water extract of hardy kiwi fruit exhibits inhibitory effect against cancer cell proliferation, high sensitivity and dose-dependent inhibition of Hep3B but with low effect on HepG2 and LoVo cell lines. The plant species *Taraxacum coreanum*, *Youngia sonchifolia* and *Ixeris dentata* belong to the family *Compositae* (commonly referred to as the sunflower family), and they are sources of natural antioxidants, mainly polyphenolic bioactive compounds (Cho et al. 2013, Chon and Kang 2013, Kang 2014). The anticancer effects of extracts prepared from these plants were investigated where they had anticancer property in the human melanoma cell lines, A375P and A375SM (Lee et al. 2016). Of the three extracts, *Ixeris dentata* had the most potent anticancer effect and inhibited tumour growth in mice without any toxicity following 4 weeks of treatment (Lee et al. 2016). More recently, it has been reported that *Ixeris dentata* extract induces apoptosis in vitro and in vivo through the phospho-Akt and phospho-nuclear factor-κB (NF-κB) signalling pathway in MDA-MB-231 breast cancer cells and tumours (Shin et al. 2017). The anticancer effects of extracts prepared from some plants of the family *Compositae*, together with their strong antioxidant activity and wide distribution throughout the world, may indicate that this family of medicinal plants can be used as potential natural source for the development of therapeutic compounds for prevention and treatment of cancer.

19.7 Conclusions

Plants or parts of plants with medicinal properties have been used for thousands of years as folk medicines in developing countries and are also a source of health benefits in developed countries. Herbal medicines offer inexpensive option and provide synergic effects with other therapies. With rapid population growth, deforestation and increasing urbanisation, protection of medicinal plants is of great concern. Utilising all plant parts, including stem, leaf, root and bark, can increase sustainability. Worthy of note that methods of conservation have been developed to overcome sustainability issues include tissue culture (Graebe and Novelli 1966), germplasm conservation (Lu et al. 1993), propagation of plants in sterile conditions (Sharma 2005) and cryopreservation (Zhang et al. 2015a).

To prevent ROS-induced oxidative damage, plants produce a very large and diverse group of antioxidants that are produced as a defence system against abiotic (light, drought, salt, heat, cold, wounding, pollutants) and biotic (pathogens, herbi-

vore) stressors (Kasote et al. 2015, Sewelam et al. 2016). Plant foods and medicinal plants are therefore important sources of antioxidants that help human to maintain a balance between pro-oxidant and antioxidant levels under physiological and pathological conditions. Decreases of endogenous antioxidants lead to oxidative stress, with long-term effects that include DNA oxidation, protein cross linking and lipid oxidation, ultimately leading to onset and progression of noncommunicable diseases. Plants are considered relatively safe, efficient and inexpensive ways of producing several valuable molecules, including many anticancer drugs. Rational food selection based on therapeutic properties and antioxidant constituents might be a useful strategy for cancer prevention (Mut-Salud et al. 2016). Although the design of drugs against cancer is one of the most difficult pharmaceutical problems, much can be achieved by intelligent application of natural and chemical principles. Natural products offer diverse molecules with high structural complexity which are essential for achieving high selectivity for cancer cells as reviewed recently (Asadi-Samani et al. 2016, Boss et al. 2016, George et al. 2017).

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Chapter 20
Antioxidants in the Prevention and Treatment of Alzheimer's Disease

Sandeep Kumar Singh, Saripella Srikrishna, Rudy J. Castellani, and George Perry

Abstract Alzheimer's disease (AD) is the most common human neurodegenerative disease that causes dementia in the elderly. A person suffering with AD shows gradual memory deterioration and other cognitive discrepancies, which eventually lead to complete incapacity and ultimately death. The major pathological characteristics of AD are the presence of senile plaques extracellularly and neurofibrillary tangles intracellularly. Growing evidences have demonstrated that oxidative stress is an important factor contributing to the initiation and progression of AD. However, the exact mechanisms that lead to the disruption of redox balance and the sources of free radicals remain elusive. The excessive reactive oxygen species may be generated from mechanisms such as mitochondrial dysfunction and/or aberrant accumulation of transition metals, while the abnormal accumulation of amyloid-β protein (Aβ) and tau proteins appears to promote redox imbalance. The resulting oxidative stress has been implicated in Aβ or tau-induced neurotoxicity. To combat oxidative stress in AD, antioxidants have been therapeutically implicated. Within the last few years, a number of polyphenolic compounds with antioxidant and neuroprotective effects have been described to possibly benefit AD patients. Many efforts have been made to explore the mechanisms behind the neuroprotective action of polyphenols. The aim of this chapter is to critically review the use of different types of antioxidants in the prevention and treatment of AD.

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20.1 Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder that causes dementia and affects middle to old-aged individuals, through the impairment of memory and cognitive function. The number of people affected with AD is expected to reach 106.8 million by 2050 (Brookmeyer et al. 2007). Around 4.5 million people are suffering from AD in the United States of America (USA) alone, and it is a major cause of death in elderly populations (Walt 2004; Korczyn and Vakhapova 2007). Regarding healthcare cost for AD patients, over US$ 200 billion is spent every year (Liu et al. 2006). AD has multiple etiological factors including environmental factors, genetics and general lifestyles (Kalaria et al. 2008). The pathophysiological hallmark of AD is extracellular deposition of amyloid-β protein (Aβ) in the forms of senile plaques and formation of intracellular neurofibrillary tangles (NFT), due to deposition of hyperphosphorylated microtubule-associated protein tau. Aβ is produced by the proteolytic processing of a transmembrane protein, Aβ precursor (AβPP) by the action of β- and γ-secretases (Wilquet and Strooper 2004). AD is usually described as a syndrome and has common clinical-pathological identities with other multiple causes. Specifically, the diagnosis of this disease is based not only on neuronal loss and memory impairment in the hippocampus area but also on deterioration in global function, reduced ability to perform day-to-day activities and the appearance of disturbance in social or occupational function. AD is mainly of two types; familial AD (FAD) and sporadic AD (SAD). FAD is caused by mutations in the AD-related genes or some of the enzymes involved in its metabolism (i.e. presenilin 1 and 2) (Selkoe 2007). However, the causes of SAD remain unclear, although it has been hypothesized that abnormal metabolic oxidation in the central nervous system (CNS) might play a key pathological role. Furthermore, the brain also exhibits reactive oxygen species (ROS) and reactive nitrogen species (RNS) mediated discrepancies which result in disturbance of normal function and metabolism of neuronal cells (Praticò and Sung 2004). ROS are very unstable and highly reactive and are generated during normal metabolic reactions. In order to have proper metabolic functioning of body cells, the levels of ROS and RNS need to be regulated by using efficient antioxidant systems.

The term antioxidant typically refers to a large and heterogeneous group of compounds that function by preventing the formation, detoxifying or scavenging of oxidant species. Antioxidants can be grouped under different categories, such as natural (e.g. plant-derived polyphenols), synthetic (e.g. butylated hydroxytoluene), vitamins (e.g. ascorbic acid, β-carotene and α-tocopherol) and inorganic (e.g. selenium). Some antioxidants act as chain-breaking molecules because they prevent the propagation of, or stop, radical chain reactions (i.e. α-tocopherol). Other antioxidants

such as superoxide dismutase (SOD) glutathione peroxidase (GPX) and catalase (CAT) detoxify superoxide radical ($^{\bullet}O_2^-$) and hydrogen peroxide (H_2O_2). These chemical reactions are crucial in cell biology because H_2O_2 in the presence of transition metals, such as iron (Fe^{2+}), generates hydroxyl radicals ($OH^{\bullet}$) which are further detoxified by reacting with non-essential molecules like oxidation of RNA that eventually degrades. In some circumstances, the production of oxidant species can exceed the endogenous antioxidant ability to destroy them and an oxidative imbalance occurs. These events result in cellular oxidative stress and subsequent molecular oxidative damage, which can translate into altered cellular functions and cell death (Halliwell and Whiteman 2004).

Taking into consideration the limits of existing preventive therapies to combat AD, intervention strategies using antioxidant-rich natural products such as fruits, vegetables and nuts are of utmost importance. The therapeutic potential of herbs and marine natural products are also considered to be preventative agents of AD. Preclinical and clinical studies have explored the neuroprotective effect of antioxidants using in vitro and in vivo models. Within the last few years, a number of polyphenolic compounds with antioxidant and neuroprotective effects have been described. Many efforts have been made to explore the mechanisms behind the neuroprotective action of polyphenols. This chapter discusses the potential neuroprotective and preventive measures of antioxidant compounds and related drugs in the prevention and treatment of AD.

20.2 Oxidative Stress in Alzheimer's Disease

AD is a neurodegenerative disorder in which oxidative stress is a key hallmark. It occurs early in disease pathogenesis and can exacerbate its progression (Nunomura et al. 2001). Several causes of oxidative stress have been determined over the years. First, the mitochondrion plays an important role in the generation and accumulation of free radicals. In addition to mitochondria, inflammation can also induce oxidative damage, especially via microglia. The latter is also important for Aβ clearance. In AD, mitochondrial function is negatively affected and the inflammatory response is implicated. Both these events lead to increased ROS formation and oxidative damage to lipid, proteins and nucleic acids.

The human CNS is rich in polyunsaturated fatty acids (PUFAs) which interact with ROS, making the CNS very prone to oxidative imbalance. The neuron, a basic function unit of the brain, has a high content of transition metals, a high metabolic oxidative rate and high ascorbate levels. Taken together, all act as potent pro-oxidants; but, on the contrary, the brain possesses a relative paucity of traditional antioxidant systems, compared with other organs. A common pathological feature of AD is the oxidation of proteins, nucleic acids and lipids in neurons (Praticò 2008).

The source of ROS species in the brain is the disturbance in proper function of mitochondria, presence of different free transition metals and Aβ peptide toxicity

(Reddy and Beal 2008). The occurrence of these processes results in combined ROS production. During the early stages of AD development, it is suggested that Aβ moves inside the mitochondria, which results in ROS production, which in turn induces oxidative stress. Furthermore, neurons also contain low levels of glutathione, a crucial antioxidant for abolishing free radicals (Pocernich and Butterfield 2012). Therefore, neurons are highly susceptible to oxidative stress.

The brains of non-demented elderly and/or SAD patients are found to be more prone to oxidative burden (Behl and Moosmann 2002; Moosmann and Behl 2002). Biomarkers for oxidative stress in the blood correspond to the same level of ROS loading in brain (Torres et al. 2007). In AD patients, many oxidative stress biomarkers (Fig. 20.1) have been identified: 4-hydroxynonenal (4-HNE) (Lovell et al. 1995; Williams et al. 2006), F2-isoprostanes (F2-IsoPs) (Praticò et al. 2000), 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), malondialdehyde (MDA) (Zhao and Zhao 2013) and protein carbonyls and 3-nitrotyrosine (Beal 2002; Butterfield and Kanski 2001). Alteration in antioxidant enzymes activity, including the modification of SOD and CAT levels, continue to contribute

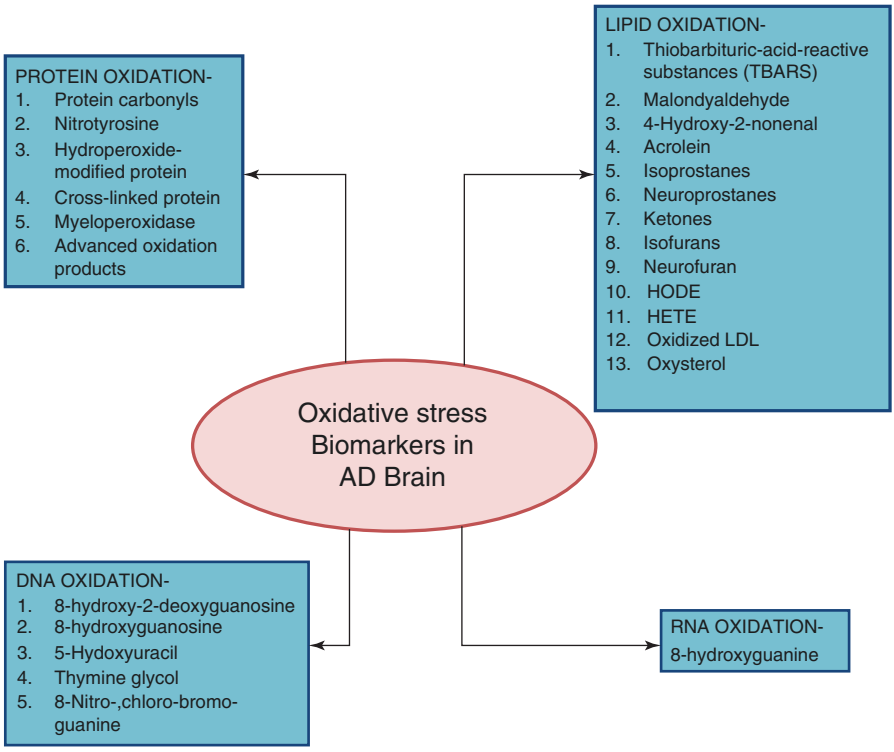


Fig. 20.1 Oxidative stress biomarkers in Alzheimer's disease (AD): the figure showing the different oxidative stress marker in case of AD including protein, lipid, DNA and RNA oxidation

towards the intracellular accumulation of free radicals in both the peripheral tissues and the CNS of AD patients (Marcus et al. 1998; Omar et al. 1999). Thus, oxidative stress is a key pathological characteristic in AD. However, the question of how and where oxidative stress originates in AD patients is still unanswered. Previous research has suggested that mitochondrial anomalies (Federico et al. 2012; Yan et al. 2013), inflammation (Lee et al. 2010; Candore et al. 2010), metal accretion (Greenough et al. 2013; Ayton et al. 2013), hyper-phosphorylation of tau protein (Stamer et al. 2002; Melov et al. 2007) and accumulation of extracellular Aβ (Chakrabarti et al. 2013) are the characteristic mechanisms for the generation of ROS and oxidative stress. The reduced activities of SOD, GPX and CAT in AD brains, contributes to the induction of brain oxidative stress (Marcus et al. 1998; Omar et al. 1999). Deficiency and the inactivity of these antioxidant enzymes hamper the clearance of free radicals. Furthermore, oxidative stress also has a very important role in the accumulation of toxic extracellular Aβ and intracellular hyperphosphorylated tau, proving that it must play a significant role in AD pathogenesis (Zhao and Zhao 2013; Yan et al. 2013; Manczak et al. 2010); therefore, it can be presumed as a biomarker and target for AD treatment (Mattson 2004; Reddy 2006; Manczak et al. 2010).

20.3 Antioxidants

Antioxidants are substances that reduce damage brought about by ROS and can delay or prevent the occurrence of anti-oxidative reactions of free radicals. Well-known antioxidants include enzymes and other substances such as ascorbic acid, α-tocopherol and β-carotene. These antioxidants are capable of counteracting the damaging effects of oxidation. Antioxidants are the substances that inhibit the oxidation process of other molecules. They abolish the oxidative reaction by eliminating free-radical intermediates from the chain reaction. Antioxidants like thiols, ascorbic acid and polyphv enols are reducing agents because they are directly oxidized during the inhibition of the oxidation reaction (Sies 1997). On the basis of occurrence, antioxidants are classified into two types: natural and synthetic antioxidants.

1. Natural Antioxidants. Natural antioxidants are classified into antioxidant enzymes and vitamins. Antioxidant enzymes such as SOD, GPX and CAT are produced by the body. Antioxidant vitamins are found in vegetables and fruits. Examples of antioxidant vitamins are β-carotenoid (vitamin A), ascorbic acid (vitamin C) and α-tocopherol (vitamin E).
2. Synthetic Antioxidants. There are many examples of synthetic antioxidants which can be taken as a food supplement. They particularly include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG) which are most commonly used worldwide.

Table 20.1 Classification of antioxidants: table listing some categories of antioxidant with few examples

Types of antioxidant	Examples
Carotenoids	β-Carotene, lycopene, lutein
Enzymes	SOD, CAT, GPX
Tripeptide	Glutathione
Hormones	Melatonin, oestrogen
Lipid-associated chemicals	Ubiquinol-10, N-acetyl cysteine, lipoic acid
Minerals	Zinc, selenium, copper
Phenolics	Quercetin, catechin
Saponines, steroids	Cortisone, estradiol, estriol
Vitamins	α-Tocopherol, ascorbic acid

Table 20.1 shows the nomenclature and classification of antioxidants, in which various antioxidants have been classified based on their structure, occurrence, solubility and kinetics. Kinetically, antioxidants can be grouped into the following six categories:

- Hydroperoxide-decomposing antioxidants: sulphide, phosphide and thiophosphate
- Metal deactivating antioxidants: diamines, hydroxyl acids and bifunctional compounds
- Antioxidants that break chains by reacting with alkyl radicals: quinones, nitrones and iminoquinones
- Antioxidants that break chains by reacting with peroxyl radicals having weak OH or N-H bonds: phenol, naphthol, hydroquinone, aromatic amines and aminophenols
- Synergism of action of several antioxidants: phenol sulphide in which phenolic group reacts with peroxyl radical and sulphide group with hydro peroxide.
- Cyclic chain termination by antioxidants: aromatic amines, nitroxyl radical and variable valence metal compounds

Furthermore, antioxidants have also been classified on the basis of their neuroprotective activity and their mode of action which includes direct, indirect and metabolic antioxidants (Behl and Moosmann 2002) mentioned in Table 20.2.

When an organism is exposed to free radicals from a variety of sources, it develops a variety of defence mechanisms (Cadenas 1997). One defence mechanism against ROS-induced oxidative stress is an enzymatic antioxidant defence system which includes SOD, GPX and CAT. In addition, there are non-enzymatic antioxidant defence mechanisms which involve compounds such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids and flavonoids. The latter, all play an important role against ROS-induced damage in an organism. During normal conditions, there is a balance between the intracellular level of antioxidants and their activity. This balance is essential for the proper health and survival of organisms.

Table 20.2 Table showing the classification of neuroprotective antioxidants on the basis of their mode of action

Direct antioxidants Compounds that chemically interfere with formed free radicals	• Aryl amines and indoles • Carotene, lycopene, retinol and other polyenes • Ebselen and other selenium-containing compounds • Flavonoids and other polyphenols • Manganese-containing mimetics of catalase/superoxide dismutase • Tocopherols and other monophenols
Indirect antioxidants Compounds that prevent the formation of free radicals	• Amino-oxidase inhibitors • Calcium antagonists • Dopamine receptor agonists • Glutamate receptor antagonists • Ion chelators • Nitric oxide synthase inhibitors
Metabolic antioxidants Compounds that limit the extent of damage to the cell by reducing the secondary metabolic burden of increased levels of free radicals	• N-Acetyl-cysteine, glutathione, 2-oxo-thiazolidine-4-carboxylate and other thiol-delivering compounds • N-Butyl-α-phenylnitrone and other spin traps • Carnitine • Creatine • Lipoic acid (thioctic acid) • Ubiquinone and idebenone

20.4 Antioxidant Defence Mechanisms

Several antioxidant defence mechanisms have evolved to protect cell components from the damage brought about by oxidative stress. The enzymes SOD, GPX and CAT are the major defences against ROS. SOD converts $^{\bullet}O_2^-$ into H_2O_2, and GPX and CAT converts H_2O_2 to molecular oxygen and water. SOD exists in two forms: Cu,Zn-SOD is present primarily in cytoplasm, while MnSOD is present in mitochondria.

The hypothesis that lifespan can be enhanced by increasing antioxidant defences has been controversial because of conflicting results in several ageing models. For example, many studies have shown that endogenous levels of antioxidant enzymes in the brain and other tissues do not decrease during ageing (Benzi and Moretti 1995; Barja 2004). Moreover, studies in mammals in which levels of antioxidants are experimentally increased have shown that maximum longevity is not affected (Harris et al. 1990; Orr et al. 2003). Experiments with *Drosophila melanogaster* have shown that overexpression of MnSOD increased lifespan (Sun et al. 2002), while overexpression of Cu,Zn-SOD had only minor incremental effects on lifespan (Orr and Sohal 1993). Similarly, enhanced levels of CAT (up to 80%) did not extend the lifespan of flies, nor did it provide improved protection against oxidative stress, induced by hyperoxia or paraquat treatment (Orr and Sohal 1992). In contrast, the concurrent overexpression of Cu,Zn-SOD and CAT was found to extend and slow down various age-related biochemical and functional alterations in *Drosophila*

(Orr and Sohal 1994). Similarly, *Drosophila* selected for longevity and *Caenorhabditis elegans* with the *age-1* mutation (a mutation associated with increased lifespan) were found to have increased activity of Cu,Zn-SOD and CAT (Larsen 1993; Dudas and Arking 1995; Hari et al. 1998).

These conflicting results suggest that an optimal balance between SOD and CAT is important for lowering the levels of oxidative stress and increasing lifespan. The effect of overexpression of SOD on lifespan has also been observed in mice (Li et al. 1995). Although several studies have shown that elevated Cu,Zn-SOD expression induced protection against oxidative stress, other reports did not find a correlation between such protection and increased lifespan. For example, homozygous transgenic mice with a two- to fivefold increase of Cu,Zn-SOD expression showed only a small increase in lifespan (Huang et al. 2000). There are many antioxidant defence mechanisms which are very important for regulation of proper function of an organism. Researchers are studying different antioxidant systems as therapeutic approaches for the prevention and treatment of human neurodegenerative diseases such as AD.

20.5 Therapeutic Approaches for the Treatment of Alzheimer's Disease

While no drug has been shown to completely protect neurons, there are two possible conceptual approaches to the treatment of AD. One approach is a treatment that prevents the onset of the disease by sequestering the primary progenitors or targets and reducing the secondary pathologies of the disease. This treatment approach would lead to a reduction in disease progression or a delay in the onset of disease, cessation or even the repair of neuronal damage after onset of disease and eventually prevention of the development of AD. Another approach is symptomatic treatment, which focuses on the tertiary cognitive symptoms of the disease and protects from further cognitive decline. This approach reflects the current state of treatment options and usually includes treating cognitive impairment, decline in global function, deterioration of ability to perform activities of daily living and behavioural disturbances. Notably, the appropriate treatment strategies depend on the severity of the disease and the specificity of each individual; however, current available therapeutic agents are mainly targeted at specific symptoms of AD.

Agents such as cholinesterase/acetylcholinesterase inhibitors (Grutzendler and Morris 2001; Ambure et al. 2014) are being used to enhance cholinergic neurotransmission and inhibition of acetylcholine degradation within the synapse for treatment of AD. Other therapeutic agents and strategies that have been studied include neurotrophins (Schulte-Herbruggen et al. 2008); antioxidants (Behl and Moosmann 2002; Moosmann and Behl 2002; Manczak et al. 2010; Chakrabarti et al. 2013); statins (Sparks et al. 2006); nonsteroidal anti-inflammatory drugs (NSAIDs) (Gasparini et al. 2004; Szekely et al. 2004; Townsend and Praticò 2005); hormone replacement therapy (Benson 1999); blocking of excitotoxicity (Braidy et al. 2010);

Aβ vaccine trials (Lemere et al. 2004); immunotherapy (Gelinas et al. 2004; Delrieu et al. 2012); metal chelator therapy (Budimir 2011; Singh et al. 2013); α-, β- and γ-secretase effectors; Aβ aggregation inhibitors; and inhibitors against Aβ-induced neurotoxicity. This chapter mainly focuses on the use of antioxidants in the prevention of AD. We will discuss in more detail the current preventive and disease-modifying strategies, using different antioxidants, for the prevention and treatment of AD among the general population.

Our knowledge of the history and pathophysiology of AD has increased greatly over the past decade, yet the causes are not clear and the cures have been subtle. Antioxidant therapy is one of the promising therapeutic strategies for AD and has been studied for years. It has been reported that antioxidants may help to prevent the cell damage caused by intracellular and extracellular $^{\bullet}O_2^-$, H_2O_2 and $OH^{\bullet}$. ROS are by-products of normal functioning cells; however, they can result in damage to cells either directly or through the activation of microglia and their role as intracellular second messengers (Staehelin 2005).

20.6 Role of Antioxidant in Treatment of Alzheimer's Disease

Currently, different natural and synthetic polyphenols are being used but are not officially approved for the treatment of AD. Among them, flavonoids are the most common group of polyphenolic compounds used in the human diet. Natural flavonoids are found ubiquitously in plants and seeds like cocoa beans and grape seeds. Flavonoids may be divided into various subclasses. A subclass, catechins are found in high concentration in the leaves of the tea plant. The major tea catechins include (−)-EC gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC) and (−)-EGC gallate (EGCG). They show complex antioxidant activity, but the main activity is the scavenging of $^{\bullet}O_2^-$ and $OH^{\bullet}$ ions by the A-ring and C-ring gallate group present in catechines (Zhu et al. 2000).

Several natural and synthetic flavonoids are a very important category of polyphenolic antioxidants. Many flavonoids are reported to have neuroprotective effects against AD (Ji and Zhang 2006; Baptista et al. 2014). A recent report from Singh et al. suggests neuroprotective role of a synthetic flavonoid derivative, revealed by in silico and in vivo studies by using a *Drosophila* model of AD (Singh et al. 2014). Furthermore, flavonoid protects neuronal cells from oxidative stress (Ishige et al. 2001) and prevents Aβ fibril formation in vitro (Hirohata et al. 2007). Flavonoid treatments also have been reported to improve learning, memory and neurocognitive performance (Vauzour 2014). Other antioxidants with neuroprotective function in context of AD include vitamins (vitamin B_{12}, vitamin E, vitamin C), hormones (oestrogen, melatonin), caffeine, berberine, silibinin (silybin), β-carotene, mitoQ, mangiferin, oleuropein aglycone, α-lipoic acid, selenium, palmatine, Ginkgo biloba, coenzyme Q10 (CoQ10), selegeiline, Szeto Schiller (SS) peptide 31, resveratrol and tea polyphenols are mentioned in Table 20.3.

Table 20.3 Name of antioxidant with chemical structure and neuroprotective function in context to Alzheimer's disease

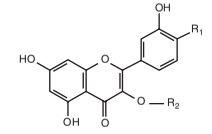
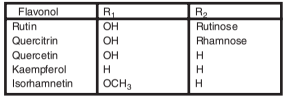
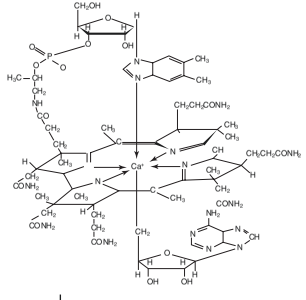
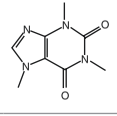
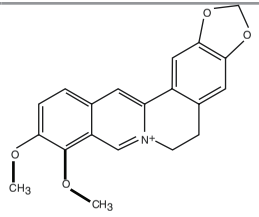
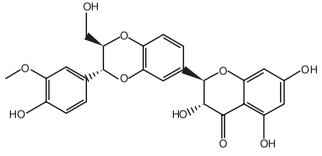
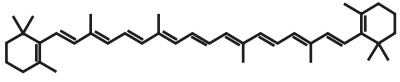
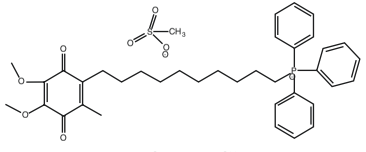
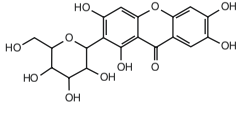
Compound name	Chemical structure	Effect/functions/biological activity	References
Flavonoids		Lower Aβ production as well as prevent Aβ fibril formation, molecular-docking study confirms direct interaction of flavonoids with amino acid residues of Aβ to prevent their self-aggregation, protect neuronal cells from oxidative stress, improve learning, memory and neurocognitive performance	Ishige et al. (2001), Ji and Zhang (2006), Hirohata et al. (2007), Baptista et al. (2014), Singh et al. (2014), Vauzour (2014)
			
	Flavonol | R_1 | R_2 Rutin | OH | Rutinose Quercitrin | OH | Rhamnose Quercetin | OH | H Kaempferol | H | H Isorhamnetin | OCH_3 | H		
Vitamin B_{12}		An antioxidant that increases choline acetyltransferase activity in cholinergic neurons in cats and improves cognitive functions in AD patients, critical in folic acid pathway and methionine homeostasis	Nadeau and Roberge (1988)
Caffeine		An antioxidant that can inhibit amyloidosis and Aβ production and reduce brain Aβ levels in transgenic mouse models for early-onset familial AD	Prasanthi et al. (2010), Laurent et al. (2014)

Table 20.3 (continued)

Compound name	Chemical structure	Effect/functions/biological activity	References
Berberine		An antioxidant that exhibits anti-AD effects through both ChEs and Aβ pathways and can also inhibit ROS and RNS	Kulkarni and Dhir (2010), Ji and Shen (2011), Durairajan et al. (2012)
Silibinin (silybin)		A flavonoid derived from the herb milk thistle (*Silybum marianum*), it prevents memory impairment and oxidative damage induced by Aβ in mice	Lu et al. (2009), Yin et al. (2011)
β-Carotene		A lipid-soluble antioxidant which may reduce lipid peroxidation, improve antioxidant status and quench singlet oxygen rapidly	Di Mascio et al. (1991), Upritchard et al. (2003)
MitoQ		An antioxidant targeted to mitochondria and is produced by conjugation of the lipophilic triphenylphosphonium (TPP+) cation to coenzyme Q	Murphy and Smith (2000)
Mangiferin		Reduces neuronal death, oxidative stress and mitochondrial depolarization, restores the GSH content (to 60% of control levels) and downregulates both SOD and catalase mRNA expression	Amazzal et al. (2007), Lemus-Molina et al. (2009), Biradar et al. (2012)

(continued)

Table 20.3 (continued)

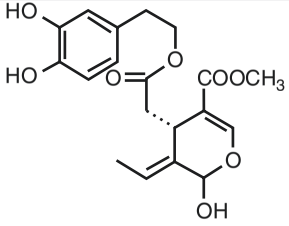
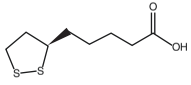
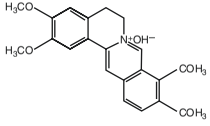
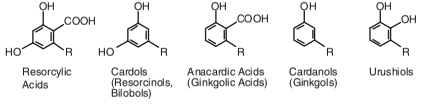
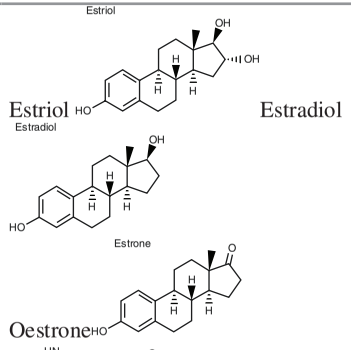
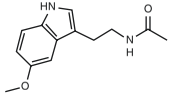
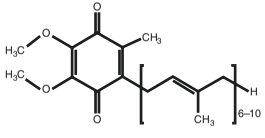
Compound name	Chemical structure	Effect/functions/biological activity	References
Oleuropein aglycone		Reduces tau aggregation, ameliorates $A\beta_{42}$-mediated toxicity	Daccache et al. (2011), Grossi et al. (2013), Luccarini et al. (2014)
LA (α-lipoic acid)		A coenzyme of mitochondrial pyruvate dehydrogenase and α-ketoglutarate dehydrogenase can recycle other antioxidants such as vitamin C and E and glutathione and increase the production of acetylcholine or as a chelator of redox-active metals to combat the accumulation of lipid peroxidation products	Ono et al. (2006), Shenk et al. (2009), Sancheti et al. (2013)
Selenium		A cofactor of glutathione peroxidase and Se-glutathione peroxidase can also act as an antioxidant	Ishrat et al. (2009a, b), Gwon et al. (2010)
Palmatine		An antioxidant that exhibits anti-AD effects through both ChEs and Aβ pathways and can also inhibit ROS and RNS	Jung et al. (2009), Dhingra and Kumar (2012)
Ginkgo biloba (different compounds present in extract)	 Resorcylic Acids; Cardols (Resorcinols, Bilobols); Anacardic Acids (Ginkgolic Acids); Cardanols (Ginkgols); Urushiols	Extract from natural plant contains many polyphenolic compounds which have ROS-scavenging ability, reduce amyloid-β production and inhibit Aβ aggregation in vitro	Stackman et al. (2003), Janssen et al. (2010), Luo et al. (2002), Vellas et al. (2012)

Table 20.3 (continued)

Compound name	Chemical structure	Effect/functions/biological activity	References
Oestrogen		Regulate glucose metabolism and mitochondrial function. Prevent Aβ-mediated toxicity in cultured neural cell. Prevent Aβ fibril formation in vitro	Kim et al. (2001), Morinaga et al. (2007), Brinton (2008), Yao and Brinton (2012)
Melatonin		A mammalian hormone synthesized mainly in the pineal gland, and it scavenges oxygen and nitrogen-based reactants generated in mitochondria by stimulating the expression and activity of glutathione peroxidase, superoxide dismutase and NO synthetase, and it also contributes to the reduction of oxidative damage in cells	Feng et al. (2006), Dragicevic et al. (2011)
CoenzymeQ10 (Ubiquinone)		A cofactor of the electron transport chain and it preserves mitochondrial membrane potential during oxidative stress and protects neuronal cells through attenuating Aβ overproduction and intracellular Aβ plaque deposits	Sadli et al. (2013)

(continued)

Table 20.3 (continued)

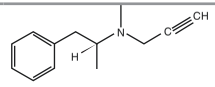
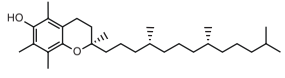
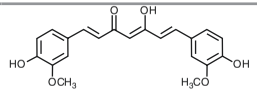
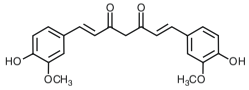
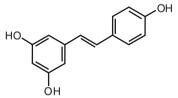
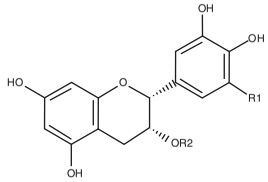
Compound name	Chemical structure	Effect/functions/biological activity	References
Selegeiline (L-deprenyl)		A selective monoamine oxidase-B inhibitor which can generate potent vasodilator nitric oxide particularly in cerebral blood vessels rapidly and protect the vascular endothelium from the toxic effects of Aβ peptide and enhance the function of nigral neurons or enhance their survival by inhibiting oxidative deamination	Gulyás et al. (2011)
Szeto Schiller (SS) Peptide 31		A mitochondria-targeted antioxidant that concentrates in the inner mitochondrial membrane and decreases mitochondrial toxicity. They can prevent the neurotoxin 1-methyl-4-phenylpyridium (MPP^+)-induced inhibition of oxygen consumption and ATP production and mitochondrial swelling	Reddy et al. (2011)
Vitamin E (α-tocopherol)		A powerful, lipid-soluble chain-breaking antioxidant found in lipid membranes, circulating lipoproteins and low-density lipoprotein (LDL) particles and has been shown to decrease free-radical-mediated damage caused by toxic chain reactions in neuronal cells and helps to inhibit dementia pathogenesis in mammalian cells	Blackett and Hall (1981), Murray and Lynch (1998), Sen et al. (2000)

Table 20.3 (continued)

Compound name	Chemical structure	Effect/functions/biological activity	References
Curcumin	 Enol Form Enol Form  Keto Form Keto Form	An antioxidant that has antioxidant, anti-inflammatory and anti-amyloid pathology activity in an Alzheimer transgenic mouse models, and it inhibits enzymes lipoxygenase and cyclooxygenase 2 that are responsible for the synthesis of the pro-inflammatory leukotrienes, prostaglandins and thromboxanes	Shih and Lin (1993), Singh and Aggarwal (1995), Nanji et al. (2003), Ono et al. (2004), Ringman et al. (2005), Nishinaka et al. (2007), Bengmark (2006), Ishrat et al. (2009a, b)
Resveratrol		Decrease production of Aβ peptide in vitro. Improve memory deficit and cognitive impairment in experimental animal models. Decrease level of MDA in animal model. Protect cells from Aβ-induced toxicity in multiple invite culture system	Virgili and Contestabile (2000), Jang and Surh (2003), Savaskan et al. (2003), Zhuang et al. (2003), Cao and Li (2004), Sharma et al. (2005), Kumar et al. (2006), Zamin et al. (2006), Ates et al. (2007), Candelario-Jalil et al. (2007), Della-Morte et al. (2009)
Tea polyphenols-(−)-epicatechin (EC) (−)-epicatechin-3-gallate (ECG) (−)-epigallocatechin (EGC) (−) epigallocatechin-3-gallate (EGCG)	 1. R1= R2 = H Epicatechin 2. R1==OH, R2 = H Epigallocatechin 3. R1 = H, R2 = galloy Epicatechin gallate 4. R1 = OH, R2 = galloyl Epigallocatechin gallate	Decrease preformed Aβ fibril in vitro, inhibit self-aggregation of Aβ aggregation. Reduce the production of Aβ peptides in AβPP695 over expressing neuron, decrease Aβ production in transgenic AD mice and reduce Aβ-induce neuronal cell death, permeable to BBB. Decrease tau pathology in transgenic AD mice and reduce Aβ-induced caspase activity in hippocampal neuronal cells	Matsuoka et al. (1995), Choi et al. (2001), Levites et al. (2001), Levites et al. (2002), Jeon et al. (2003), Lee et al. (2003), Rezai-Zadeh et al. (2005), Bastianetto et al. (2006), Obregon et al. (2006), Ehrnhoefer et al. (2008)

Vitamin B_{12} supplementation significantly affects the acetylcholine transferase activity in cat brain (Nadeau and Roberge 1988). Caffeine protects rabbit hippocampus by preventing oxidative stress induced by cholesterol-rich diet (Prasanthi et al. 2010). Caffeine has also been reported to have beneficial effects on AD model of tau pathology (Laurent et al. 2014) and reduces Aβ production in a mouse model of AD (Chu et al. 2012; Laurent et al. 2014). Berberine is a plant alkaloid, reported to have an important therapeutic role in central nervous system disorders such as AD (Kulkarni and Dhir 2010; Ji and Shen 2011). In mouse models of AD, berberine prevents Aβ pathology and cognitive impairment (Durairajan et al. 2012).

Another important antioxidant, silibinin plays an important role against ROS production and Aβ aggregation inhibitor (Lu et al. 2009; Yin et al. 2011). β-Carotene is a lipid-soluble antioxidant which may reduce lipid peroxidation, improve antioxidant status and quench singlet oxygen rapidly (Di Mascio et al. 1991; Upritchard et al. 2003). An antioxidant targeted to mitochondria is mitoQ which is produced by conjugation of the lipophilic tetraphenylphosphonium cation (TPP+) to coenzyme Q (Murphy and Smith 2000). Mangiferin is reported to prevent oxidative stress and neuronal cell death and to regulate the SOD and CAT expression (Amazzal et al. 2007; Lemus-Molina et al. 2009; Biradar et al. 2012). Oleuropein aglycone acts as tau aggregation inhibitor as well as prevents Aβ-aggregation (Daccache et al. 2011; Grossi et al. 2013; Luccarini et al. 2014). Lipoic acid treatment modulates synaptic plasticity in a mouse model of AD (Sancheti et al. 2013), improves cognitive functions in apolipoprotein E mice (Shenk et al. 2009) and exhibits anti-amyloid activity in vitro (Ono et al. 2006). Selenium is a very important cofactor of GPX that acts as an antioxidant to prevent Aβ-toxicity (Ishrat et al. 2009a, b; Gwon et al. 2010). Palmatine has been reported to have antioxidant and anti-AD effects and can also inhibit ROS and RNS formation (Jung et al. 2009; Dhingra and Kumar 2012).

Ginkgo biloba is a tree; its extract contains many polyphenolic compounds having free-radical scavenging capability (Janssen et al. 2010). Ginkgo extract helps to prevent age-related spatial memory deficits in transgenic mouse model of AD and inhibits Aβ-aggregation and caspase-3 activation (Luo et al. 2002; Stackman et al. 2003; Vellas et al. 2012). Oestrogen and melatonin hormones also play an important role in regulation of glucose metabolism and proper mitochondrial function (Brinton 2008; Yao and Brinton 2012). These hormones have a neuroprotective role against Aβ-mediated toxicity in neuronal cell culture (Kim et al. 2001) and anti-amyloidogenic effect on Aβ fibril in vitro (Morinaga et al. 2007). Melatonin also restored mitochondrial function (Dragicevic et al. 2011) and reduced oxidative stress in a transgenic mouse model of AD (Feng et al. 2006).

CoQ10 is a cofactor of the electron transport chain, and it prevents Aβ overproduction and its deposition intracellularly (Sadli et al. 2013). Selegeiline is a monoamine oxidase-B inhibitor which can protect the vascular endothelium from toxic effects of Aβ peptide and promote function of nigral neurons (Gulyás et al. 2011). Szeto Schiller (SS) peptide 31 is a mitochondrial-targeted antioxidant (MTA) that

localizes to the inner mitochondrial membrane and decreases mitochondrial toxicity. It can also prevent mitochondrial swelling by preventing neurotoxin 1-methyl-4-phenylpyridinium (MPP^+)-induced inhibition of oxygen consumption (Reddy et al. 2011).

20.7 Antioxidant Therapy Development for Alzheimer's Disease

The stages of free-radical production may be arbitrarily divided into (1) conditions prior to their formation, (2) free-radical formation and (3) adduction. The different types of antioxidant therapies are categorized according to their intervention at specific points in the stages of free-radical formation. Summarized below are current strategies for developing antioxidant therapy for AD.

20.7.1 Vitamin E

Vitamin E is the most commonly administered antioxidant supplement, and studies indicate it increases longevity of short-lived species such as *C. elegans* and the rotifer *Asplanchna brightwelli* (Sawada and Enesco 1984; Harrington and Harley 1988), but less evidence is available for an increased lifespan in rodents (Blackett and Hall 1981; Morley and Trainor 2001). Evidence for functional lifespan improvement (i.e. improvements of brain physiology and health) is more compelling. For example, vitamin E, when administered orally to rodents, can ameliorate age-related impairments in long-term potentiation (Murray and Lynch 1998) and improve memory and cognitive behaviours (Joseph et al. 1998, 1999). It is also reported that vitamin E can be used to inhibit c-Src kinase and death of HT4 neuronal cells (Sen et al. 2000).

Vitamin E has a positive impact on the prevention of neurodegenerative disease in vitro and in apolipoprotein E animal model studies. For example, vitamin E has a neuroprotective role in apoE-deficient mice (Veinbergs et al. 2001) and reduced Aβ-induced neurotoxicity in cultured hippocampal neurons (Butterfield et al. 1999). In addition, in vivo studies have demonstrated the neuroprotective activity of vitamin E through the prevention of Aβ toxicity (Yamada et al. 1999). In the latter, vitamin E prevented memory and behavioural deficits induced by infusions of synthetic Aβ into the cerebroventricles. Despite this, the data obtained from clinical trials in humans are less convincing; vitamin E is unable to establish improvements in cognition in AD patients; little improvement in living performance has been observed (Sano et al. 1996, 1997). Different epidemiological studies have not always shown a positive link between increased intake of vitamin E and decreased incidence of AD (Lonn et al. 2005). Some studies have shown a correlation between dietary intake of vitamin E-rich food, like fruits and

vegetables, and a decreased incidence of AD (Lee et al. 2005). This indicates a possible role of diets rich in vitamin E and other phytochemicals in AD prevention.

20.7.2 Polyphenolic Compounds

Vegetables, fruits, nuts and other whole foods contain thousands of polyphenolic compounds with antioxidant and anti-inflammatory properties (Annapurna et al. 1991; Careri et al. 2001; Zhang et al. 2002; Reddy et al. 2003). Plants provide a wide variety of polyphenols that act in synergy to promote human health. Nutritional neuroscience is an area of research that is rapidly growing in popularity, and there are ongoing studies exploring the effects of polyphenols in foods and herbs as neuroprotective agents.

Polyphenols have been shown to be effective against certain chronic diseases, such as cardiovascular and cancer diseases, protecting body organs and tissues against oxidative stress (Manach et al. 2005; Duthie 2007). The antioxidant properties and health-promoting activity of polyphenols can explain the potential of these plant-derived compounds in the prevention and treatment of AD (Singh et al. 2008). Plant-derived polyphenols are the most plethoric dietary antioxidants; however, numerous experimental studies performed in cell culture and various animal models have demonstrated that the antioxidant activity of these compounds is unlikely to be the sole explanation for their protective cellular effects. Among the different types of polyphenols, researchers are focusing on curcumin, resveratrol and green tea catechins. Epidemiological studies suggest that a positive relationship exists between consumption of these phytochemicals and the prevention of AD (Singh et al. 2008). In particular, resveratrol (trans-3, 4′,5-trihydroxystilbene), curcumin (diferuloyl methane) and (−)-epigallocatechin gallate (EGCG) exhibit antioxidant properties as well as anti-amyloidogenic activity, which reduces Aβ fibril formation (Ono et al. 2004; Marambaud et al. 2005; Rezai-Zadeh et al. 2005). In the following sections, we focus on use of selected plant-derived polyphenols for their antioxidant properties that might be capable of slowing AD progression (Table 20.3).

20.7.2.1 Curcumin

Curcumin is a yellow-pigmented chemical isolated from the rhizome of turmeric (*Curcuma longa*). It is used chiefly for food preservation and as a spice to add a specific flavour to Indian curries (Ringman et al. 2005). Interestingly, it was reported that the prevalence of AD in aged people (70–79 years) in India is 4.4-fold less than in the USA, suggesting that it might be due to a regular consumption of curcumin-rich diets by the Indian population (Ganguli et al. 2000). Different in vitro and in vivo studies show that curcumin has anti-oxidative, anti-amyloidogenic and

anti-inflammatory properties (Zhao et al. 1989). One study by Ono et al. demonstrated that rosmarnic acid (an analogue of curcumin) has the same anti-amyloidogenic properties as curcumin; supplementation of both these compounds significantly inhibits formation of Aβ aggregates and also destabilizes preformed Aβ fibrils (Ono et al. 2004).

Preventing Aβ fibril formation is a very promising therapeutic strategy for AD treatment; thus, inhibition of Aβ fibril formation by curcumin is a possible agent for AD prevention and/or treatment. Although the mechanism regarding its anti-amyloidogenic property is not clear, it has been suggested that the complex structure of curcumin, which has a short carbohydrate chain binding two 3,4-methoxyhydroxyphenyl rings, might be capable of binding-free Aβ and subsequently inhibiting Aβ fibrillization (Ono et al. 2004). Alternatively, curcumin may specifically interact with Aβ fibrils and destabilize the β-sheet conformation of Aβ fibrils (Ono et al. 2004). Curcumin possesses strong antioxidant activity, a property that led to its early use as a food preservative (Ringman et al. 2005). According to Zhao et al. (1989), curcumin has much stronger free-radical scavenging activity than vitamin E; in particular, it targets NO-based radicals and scavenges them. This activity protects the brain from lipid peroxidation (Wei et al. 2006). It also prevents oxidative damage of DNA in mouse fibroblasts by the $OH^{\bullet}$ scavenging to prevent 8-hydroxy-2-deoxyguanosine formation within DNA molecule (Shih and Lin 1993).

Curcumin has been shown to bind with some metal ions like Cu^{2+} and Zn^{2+} under physiological conditions (Baum and Ng 2004). These metal ions play an important role in aggregation of Aβ by binding with metal ion domains present in Aβ sequence, leading to oxidative damage in the AD brain. Curcumin may prevent redox metal ion-mediated Aβ neurotoxicity in AD brains (Baum and Ng 2004). Curcumin also plays an important role in glutathione S-transferase activation (Nishinaka et al. 2007) and helps to partially restore glutathione content in brain tissue (Ishrat et al. 2009a, b). Further, evidence for the antioxidant property of curcumin is reported by an experimental study performed by Lim et al. (2001) in a transgenic mouse model of AD. In the latter experiment, curcumin was fed to AD mice, resulting in a reduction of oxidized proteins containing carbonyl groups. Thus, curcumin reduced the brain levels of oxidized proteins containing carbonyl groups.

It has been reported that curcumin suppresses the activity of the transcription factors, Nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1), and regulates the inflammatory response (Singh and Aggarwal 1995; Nanji et al. 2003; Sandur et al. 2007; Shishodia et al. 2007; Bengmark 2006). Curcumin is efficiently able to block the induction of inducible nitric oxide synthase (NOS), probably by inhibiting NF-κB activation (Nanji et al. 2003; Sandur et al. 2007; Bengmark 2006). Interestingly, curcumin has been shown to abolish Aβ-induced expression of different cytokines and chemokines in both THP-1 monocytic cells and peripheral blood monocytes (Giri et al. 2004). Lim et al. (2001) showed that curcumin significantly reduced the level of Interleukin 1 beta IL-1β in Tg2576 AD-like mice. Several animal models of AD have been used to show the anti-AD effects of curcumin. When curcumin was fed to Aβ-injected rats, they exhibited improved mem-

ory and brain function compared to Aβ-injected rats alone (Frautschy et al. 2001). In addition, when curcumin was fed to aged Tg2576 mice, the same effect was reported (Lim et al. 2001). There is also proof that curcumin can cross the blood-brain barrier to target senile plaques and dissolve already formed senile plaques in mouse models of AD (Yang et al. 2005; Garcia-Alloza et al. 2007). These observations obtained by previous experimental studies in various model systems support the theory that dietary supplementation of curcumin could be an effective mean to prevent or treat AD.

20.7.2.2 Resveratrol

Resveratrol, a powerful polyphenol, is mainly found in red grapes, peanut butter, dark chocolate, Itadori tea and blueberries. It has been reported that resveratrol has a wide range of biological and pharmacological activity (Soleas et al. 1997). An inverse relationship between wine consumption and incidence of AD has been observed (Orgogozo et al. 1997; Lindsay et al. 2002), leading to the hypothesis that resveratrol might be the underlying factor behind reported beneficial effects of wine on AD patients. Resveratrol is similar to curcumin in its ability to cross the blood-brain barrier and penetrate brain tissue (Virgili and Contestabile 2000; Wang et al. 2002). In vitro and in vivo studies in several model systems have shown the neuroprotective effect of resveratrol (Jang and Surh 2003; Zamin et al. 2006; Ates et al. 2007; Della-Morte et al. 2009).

An anti-amyloidogenic effect of resveratrol is reported in several cell lines expressing Swedish mutant AβPP695 by reducing the level of secreted or intracellular Aβ peptides; however, it does not affect the serine proteases, β- or γ-secretase (Marambaud et al. 2005). Several lines of experimental studies suggest that resveratrol may prevent oxidative stress involved in AD pathogenesis, making resveratrol a promising therapeutic agent to prevent or treat AD. As curcumin, resveratrol has scavenging abilities and protects neurons and microglial cells (Savaskan et al. 2003; Zhuang et al. 2003; Cao and Li 2004; Candelario-Jalil et al. 2007). Resveratrol also upregulates cellular antioxidants, including glutathione, induces the gene expression of phase 2 enzymes and protects against oxidative and electrophilic injury (Sharma and Gupta 2002). In the brain of rats, oxidative stress induced by streptozotocin or the microtubule-disrupting agent, colchicine, was reduced by the neuroprotective action of resveratrol. Regular administration of resveratrol in rats also led to a significant reduction in the elevated levels of MDA (Sharma et al. 2005; Kumar et al. 2006). Improvement in memory and cognitive function by supplementation of resveratrol has been revealed in various experimental animal models. Trans-resveratrol seems to improve cognitive dysfunction and spatial memory deficits (Sharma and Gupta 2002; Sharma et al. 2005; Kumar et al. 2006). The latter studies and other experimental observations suggest that resveratrol has antioxidant, chemopreventive and/or neuroprotective activity and could be a suitable therapeutic agent for prevention and treatment of AD.

20.7.2.3 Green Tea Catechins

Green tea catechins (GTCs) consist mainly of four different epicatechin derivatives: epicatechin (EC), epicatechin gallate (ECG) epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Catechin supplementation has been associated with a variety of beneficial health effects in human beings. GTCs can easily cross the blood-brain barrier (Nakagawa and Miyazawa 1997; Suganuma et al. 1998; Mandel et al. 2006). Recent studies describe a neuroprotective role of GTCs (Rezai-Zadeh et al. 2005, Singh et al. 2008), together with several molecular biological roles, including activation of protein kinase C, MAPKs, antioxidant enzymes and survival genes, calcium homeostasis and AβPP processing (Chen et al. 2000; Levites et al. 2001; Mandel et al. 2006). Several in vitro studies have clearly demonstrated the neuroprotective effect of green tea extract on Aβ-induced neurotoxicity (Levites et al. 2001, 2002).

EGCG, a major component of green tea, decreased Aβ levels and plaques in Tg2576 AD mice and reduced Aβ generation in AβPP695-overexpressing neurons (Rezai-Zadeh et al. 2005). These effects were linked with elevated activity of α-secretase and thus increased generation of the α-C-terminal fragment of AβPP and the soluble fragment of amyloid precursor protein-α (sAβPPα), indicating that EGCG has an important role in the regulation of the non-amyloidogenic pathways of AβPP processing (Levites et al. 2001; Rezai-Zadeh et al. 2005). In addition, EGCG interestingly increased the protein level of active α-disintegrin as well as metalloprotease 10, both of which are candidates having important roles in α-secretase activity, which ultimately leads to non-amyloidogenic AβPP processing (Choi et al. 2001). Further, evidence on β-secretase inhibitor activity of EGCG in a cell-free system has been sustained through the demonstration of Aβ generation prevention, brought about by the EGCG-blocking β-secretase activity (Bastianetto et al. 2006). GTCs are also able to prevent the formation, self-interaction and stabilization of Aβ fibrils (Ehrnhoefer et al. 2008). EGCG has been shown to efficiently inhibit Aβ aggregate formation by directly interacting with Aβ peptide through stable hydrogen binding and inhibiting formation of toxic Aβ aggregates (Matsuoka et al. 1995).

Overall, catechins have potentially more antioxidant properties than vitamins C or E (Zhao et al. 1989). Another potent example of catechins' antioxidant activity is chelating metal ions like copper, zinc and iron preventing free-radical generation (Singh et al. 2008). In addition, ROS scavenging activity of EGCG and inhibition of lipid peroxidation are well reported (Choi et al. 2001). EGCG significantly decreases caspase activity and MDA levels in cells exposed to Aβ; this reduction results in protection against Aβ-induced apoptosis and enhances neuronal survival in the hippocampus (Choi et al. 2001). In an in vivo experiment, EGCG was able to inhibit Aβ-mediated oxidative stress and reduce hippocampal lipid peroxidation in rat (Choi et al. 2001). EGCG modulates Aβ-mediated tau pathology in Tg2576 mice, reducing potentially toxic sarkosyl-soluble phospho-tau isoforms (Bastianetto et al. 2006). In a recent study, Heat shock protein 90 (HSP90) inhibitors were found to reduce levels of soluble phospho-tau isoforms (Obregon et al. 2006). EGCG has

been found to directly bind HSP90 and inhibit its activity (Jeon et al. 2003); thus EGCG might modulate the level of phospho-tau through the inhibition of HSP90. EGCG also possesses anti-inflammatory properties through inhibition of NFκB and MAPK and attenuation of IL-6, IL-8 and endothelial growth factor (EGF) production in human astrocytoma U373MG cells (Ono et al. 2003). It has also been reported that inflammatory activities of various cytokines, suppressed by EGCG, attenuate prostaglandin E2 production, IL-1- and Aβ-induced COX-2 expression (Ono et al. 2003).

EGCG has been found to reduce cognitive impairment in Tg2576 mice (Bastianetto et al. 2006). In another study, rats were provided with drinking water containing high levels of green tea catechins (mostly EGCG) for 5 months; these rats showed less memory impairment following intracerebroventricular injection of $A\beta_{1-40}$ than those supplied normal drinking water (Levites et al. 2001). In a mouse model of cerebral ischemia, injection of tea catechins ameliorated hippocampal neuronal damage and memory impairment (Matsuoka et al. 1995; Lee et al. 2003). Treatment with green tea catechins, especially EGCG, might be a viable therapeutic approach for treating AD-like brain neuropathologies and associated cognitive impairment.

20.8 Conclusions

AD is a common human neurodegenerative disease with a complex and long asymptomatic period prior to placeable clinical dementia. It has been well established that oxidative damage of cellular molecules plays a significant role in neurodegenerative disorders such as AD, which may result in neuronal cell death. Previous studies on AD mouse models showed that increased oxidative damage is a comparatively early event in the pathogenesis of AD that may be inhibited by antioxidants. Therefore, the use of differntes types of antioxidants, which prevent or reduce oxidative damage, may provide ptential therapeutic strategies aiming at prevention and treatment of AD. The AD mouse models as well as small clinical studies show evidence for AD treatment through the use of mitochondria-directed antioxidants, metabolic antioxidants and Szeto Schiller (SS) peptides. Some previous studies also show that treatment with different antioxidants such as vitamin C, vitamin E, oestrogen, selegiline, *Ginkgo biloba,* etc. may be effective therapy for AD to some extent; however, this positive effect in clinical patients is still controversial.

Many studies using cell lines and animal models suggest an important role of antioxidants in AD prevention and treatment. However, these antioxidants are less effective in human clinical trials. Most of the currently known antioxidants are limited in their ability to cross the blood-brain barrier. Therefore, development of smaller antioxidant molecules that would easily pass through blood-brain barrier offer more promises for antioxidant drug development for AD treatment. Additionally, it is imperative that future trials utilize combinations of antioxidants, rather than a single one. This might help with facilitating redox cycling, along with

maximizing bioavailability to different cellular compartments of an organism. Further clinical studies are required to determine if antioxidants may reduce or prevent the risk of AD development and progression.

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