Dietary carotenoid roles in redox homeostasis and human health.

Marcelo P. Barros[1,2,*], Maria J. Rodrigo[2], Lorenzo Zacarias[2].

[1]Institute of Physical Activity and Sports Science (ICAFE), Cruzeiro do Sul University, Rua Galvao Bueno 868, Building B, 13th floor, 01506000, Sao Paulo, SP, Brazil.

[2]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Departamento de Biotecnología de los Alimentos, Calle Catedrático Agustín Escardino 7, 46980 Paterna, Spain.

*Corresponding author (current address)

Marcelo Paes de Barros (cited as *Barros, M.P.*)

Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Departamento de Biotecnología de los Alimentos, Calle Catedrático Agustín Escardino 7, 46980 Paterna, Spain.

Emails: marcelo.barros@cruzeirodosul.edu.br

marcelobarros-iata@csic.es

Abstract

Classic nutrition believed that healthy diets should simply provide sufficient antioxidant loads to organisms, to hamper free radical processes and avoid oxidative stress. Current redox biology was proven much more intricate. Carotenoids are bioactive compounds in human diet with a multifaceted role in redox metabolism. This review discusses the participation of α/β-carotene, lutein, zeaxanthin, lycopene, β-cryptoxanthin, astaxanthin, and derivatives in redox homeostasis focusing on: (i) their anti-/pro-oxidant activities; (ii) control of gene expression via Nrf2-Keap1 and NF-κB pathways; and (iii) their link with (sub)cellular redox circuits, as part of the 'redox code' that orchestrates physiological processes and health in humans.

Keywords

Oxidative Stress – Antioxidant – Nrf2 – Free Radicals – Apocarotenoid – Inflammation – Apoptosis – Redox Switch

Manuscript text

Introduction

Many bioactive compounds in human diet were shown to be associated with good health conditions and prevention of many diseases, including atherosclerosis, cardiopathies, cancer (e.g., skin, mammary, and prostate), retinopathies, and neurodegenerative disorders, like Parkinson`s and Alzheimer`s diseases. In the last decades, many studies reported health benefits associated with consumption of ascorbic acid (vitamin C), α,γ-tocopherols (vitamin E), folic acid, flavonoids, mono- and polyunsaturated fatty acids, anthocyanins, sulfur-rich compounds (e.g. allicin), and carotenoids. However, more recent findings have shown that rather than simply scavenging free radicals and avoiding oxidation of biomolecules, the participation of these dietary compounds in redox metabolism is by far more complicated. The cellular redox homeostasis is sustained by an overall (integrative) and well-adjusted net of subcellular redox circuits that constantly oscillate depending on nutrient and energy supplies, genetic and epigenetic codes, and interactions with the external (micro)environment. Carotenoids are particularly intriguing bioactive compounds of human diet, based on the varied mechanisms they interact with those redox circuits.[1]

Sources of carotenoids

Of the more than 700 carotenoids and xanthophylls (oxygen-containing carotenoids) already identified, seven of these are more frequently found in human diet: α/β-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin, and astaxanthin.[1] A wide variety of plant

foods (vegetables and fruits), maintained throughout the year, represent the main sources of carotenoids for humans. Green fruits (green pepper), green leafy (e.g. lettuce, spinach) and non-leafy vegetables (e.g. artichokes, green asparagus) provide substantial amounts of lutein and minor proportions α/β-carotene and zeaxanthin. Tomato and derived cooked products (baked, stuffed or fried) are the main sources of lycopene, despite also providing significant quantities of β-carotene, phytoene and phytofluene. Orange and yellow-colored fruits, seeds and non-leafy vegetables (mainly roots) are also significant dietary sources of α/β-carotene, zeaxanthin and β-cryptoxanthin. Carrot is the main contributor to α/β-carotene, but seasonal intake of other orange-colored fruits like peach, apricot and pumpkin are also good sources of these carotenes in diet. The main sources of β-cryptoxanthin in human diet are sweet oranges and mandarins. This list also includes violaxanthin and neoxanthin (chloroplast-associated carotenoids), the two xanthophylls from red peppers and paprika, capsanthin and capsorubin (although with low absorption in humans), and the colorless carotenes phytoene and phytofluene, which, despite absorbed, have not been extensively investigated for their nutritional/physiological effects.[1,2] Although fruits and vegetables are undeniably the main sources of carotenoids in human diet, we cannot ignore the contribution of animal-derived foods, such as egg yolk (accounting for important zeaxanthin, lutein and α/β-carotene sources), dairy products (for α/β-carotene), and salmonid fishes/seafood (main sources of astaxanthin and canthaxanthin). No specific recommendation for daily total carotenoid intake has been officially issued by any nutritional/health organization so far, *e.g.* US Food and Drug Administration (FDA) or World Health Organization (WHO), but, based on the Recommended Dietary Allowance (RDA) for retinol equivalents in healthy adult males (700-900 μg retinol equiv./day), a

Prudent Individual Daily Intake (PIDI) was assumed around 9.0 to 18.0 mg total carotenoid/day.[1] Table 1 presents data from National Surveys of carotenoid intakes in Brazil, Spain, and United States, statistical information about the surveys, and the Prudent Individual Daily Intakes (PIDI) for main dietary carotenoids.[1,3]

Bioavailability and absorption

The transfer of carotenoids from food stuff to target tissues and cells depends, obviously, on many factors, including bioavailability from the food matrix, chemical transformation during digestion (carotenoid ester hydrolysis, *cis-trans* isomerization, etc.), absorption by the gastrointestinal tract, and transport to different human cells and tissues.[4] Moreover, recent findings indicate that host-gut microbiota interactions could afford variable interindividual carotenoid absorption, specially through changes in bile acid/salt composition and concentration, and via carotenoid efflux back to intestinal lumen by scavenger receptor-class B type I (SR-BI).[5]

Carotenoids absorption in enterocytes occurs by passive diffusion and by active transport mediated by the membrane proteins, SR-BI, Niemann-Pick C1-Like 1 (NPC1L1), and cluster determinant 36 (CD36).[5] The complexity of carotenoid absorption, transport, and accessibility to specific tissues are hard controlling factors to establish a clear dose-response relationship between the amounts of carotenoids in foods and the physiological effects on human health and disease prevention. Therefore, integrative-translational strategies are necessary to compile and process this information. Recent advances in the "omics" techniques (i.e., genomics, transcriptomics, proteomics, and metabolomics) have

enabled a more rapid and comprehensive understanding of the biological processes that link carotenoid nutrition and human health.[6] Moreover, several studies have clearly shown that individually expressed genetic polymorphisms determine the distinct capacities to uptake and metabolically process macro- and micronutrients (including vitamin → cofactor transformations), activate phosphorylation-dependent signaling pathways, and respond to hormone variations.[7] Not surprisingly, lifestyle (exercising, smoking, etc.), the environment (including social/emotional stress), and even cultural feeding habits of individuals can switch on/off the expression of a multitude of genes, from the adaptive to the housekeeping genes, thus revealing an epigenetic code that may modulate the genetic and histone-controlled basis of molecular information.[8] Nutrigenetics and nutrigenomics have generated recurrent and controversial discussions about personalized nutrition versus the Reference Daily Intake (RDI) recommendations, especially in terms of disease prevention and recovery, when metabolic and oxidative stresses are extreme.[9]

Metabolism

Within enterocytes, dietary carotenoids may be cleaved by 15,15′-oxygenase 1 (BCO1) and/or 9′,10′-oxygenase 2 (BCO2), despite differences in enzyme/substrate affinities (K_m values), resulting in formation of a wide range of apocarotenals and retinol-derived products (only from pro-vitamin A carotenoids). Although multiple apocarotenoids may be found in circulation after absorption, most carotenoids are found intact in chylomicrons and other circulating lipoproteins after regular and carotenoid-rich meals.[4] Further hepatic (oxidative) metabolism also produces epoxy-, hydroxy-, and keto-derivatives of original dietary carotenoids that are also found in circulation. More

information about sources, absorption, bioavailability, and metabolism of dietary carotenoids is currently available in a recent review authored by Rodriguez-Concepción et al. 2018.[1]

Modes of Action

The health benefits provided by dietary carotenoids foundationally rely on five biological properties: (i) as antioxidants that scavenge and quench reactive redox intermediates of oxidative metabolism; (ii) as electrophiles that enhance endogenous antioxidant systems; (iii) as pro-vitamin A compounds that trigger retinol-mediated pathways; (iv) by suppressing inflammation-related processes mediated by nuclear factor *kappa*-light-chain-enhancer of activated B cells (NF-κB) pathway; and/or (v) by directly bonding nuclear receptors (NR) and other transcription factors in target cells.[10]

Retinoic acid and apocarotenoids like apo-10'-lycopenoic acid and β-apo-14'-carotenal are ligands of the canonical retinoid acid-receptors (RARs) and retinoid X receptors (RXRs) which affects the expression of a vast array of responsive genes involved in cholesterol, fatty acid, Ca^{2+}, and phosphate homeostasis (tissue-dependent affinity), accounting for general organism development.[11] Some aldehyde-derivatives of metabolized carotenoids also show activity in Peroxisome Proliferator-Activated Receptors (PPARs)-triggered genes, mostly by molecular interactions with Activator Protein-1 and/or CCAAT-enhancer binding Proteins (AP-1 and C/EBPs, respectively), which are key components of these signal cascades.[1,10] Other putative contributions of carotenoids to human health

involve inhibiting tumor cell proliferation (not necessarily mediated by redox species) and improving intercellular communication at gap junctions.[1]

In general, studies with mammals have shown a close link between carotenoid nutrition and hepatic oxidative and lipid metabolism. Using a mouse model, Palczewski et al. (2016) screened 30,855 genes in a hepatic transcriptome of carotenoid-fed mice and observed changes in the expression of 1,207 genes (approximately 4%) between β-carotene-treated animals and control mice, and 2,133 genes (~7%) in the zeaxanthin group versus its control group.[12] Interestingly, several of the differentially expressed genes were involved in lipid metabolism, energy metabolism, and mitochondrial redox homeostasis. In another study with HepG2 cells and Chinese hamster ovary cells, astaxanthin supplementation regulated many PPAR-responsive genes in cellular lipid and glucose metabolism pathways, mainly via the glycolysis and tricarboxylic acid cycle pathways and normalized the hepatic transcriptome profile induced after lipid loading in hepatocytes. The effects were believed to ameliorate the clinical symptoms of hyperlipidemia, insulin resistance, and obesity.[13] Hierarchical clustering (transcriptomic) analysis of 202 genes from human breast cancer cells MCF-7 cells treated with lycopene accordingly revealed carotenoid regulation on apoptosis, cell cycle and DNA repair mechanisms.[14]

Carotenoids in redox metabolism

Anti- and pro-oxidant properties of carotenoids

As free radical scavengers, carotenoids react with reactive oxygen/nitrogen species (ROS/RNS) by three distinct mechanisms: (i) radical addition/adduct formation; (ii)

electron transfer; and (iii) allylic hydrogen abstraction (further details in the classic paper of Young & Lowe, 2001).[15] However, their antioxidant properties were shown to change with membrane lipid composition, free radical species, pH, temperature, and pO_2.[15,16] Many studies have also shown that carotenes and xanthophylls have pro-oxidant properties under some circumstances, e.g. when lipid peroxidation is in progression under high pO_2. Under these experimental conditions, a higher proportion of carotene-peroxyl radical (Car-OO$^{\bullet}$) is formed and, unless efficiently eliminated by other antioxidant systems, Car-OO$^{\bullet}$ will propagate lipid peroxidation by further attack on intact unsaturated fatty acid chains in membranes.[15] Incorporation of carotenes and xanthophylls into membranes result in spatial peculiarities that affect packing and fluidity of lipid bilayers.[16] More rigid membranes are more prone to structural fractures and integrity loss (e.g. when overloaded with polar xanthophylls), whereas too fluid membranes are more susceptible to free radical access within the hydrophobic core leading to higher oxidation (e.g. membranes enriched with omega-3 polyunsaturated fatty acids).[17] Synergistic antioxidant activity with other carotenoid/xanthophyll molecules or in the presence of water-soluble antioxidants, such as ascorbic acid, have been extensively described in literature.[15]

Because most carotenoids have limited absorption and tissue-specific accumulation *in vivo*, it is inaccurate to assume that these compounds will promote health simply by removing ROS/RNS *in situ*. Indeed, many studies have shown that most bioactive dietary compounds (except the α,γ-tocopherols) could not fully exert their antioxidant activities *in vivo*, due to kinetic limitations imposed by their low scavenging rate constants compared to the reactivity of most damage-promoting radicals, e.g., HO$^{\bullet}$, RO$^{\bullet}$, and ROO$^{\bullet}$ ($k_1 > 10^7$ $M^{-1}s^{-1}$)[18] Instead, enzymatic removal of nonradical electrophiles, such as lipid hydroperoxides

(LOOH) and H_2O_2, in two electron redox reactions appears to be the major antioxidant mechanism *in vivo*.[19] Although we cannot ignore the inherent scavenging properties of carotenoid compounds, their most significant contribution to cell protection is assumed to be inducing endogenous antioxidant defenses (through Nrf2-Keap1 pathway) and quenching singlet oxygen ($^1O_2$), by dissipating its excitatory energy as heat.[1,18-20]

Redox homeostasis

Redox homeostasis encompasses the participation of nucleophile compounds, namely glutathione (GSH) and thiol(SH)-dependent redoxins, the reductase enzymes of their oxidized forms (e.g., glutathione reductase, GR, which reduces oxidized glutathione, GSSG → 2GSH), and the efficient supply of reducing equivalents from NADPH throughout the pentose phosphate pathway.[18] Among all thiol(SH)-components, GSH undoubtedly plays a pivotal role in sustaining redox homeostasis, but it is also central in cellular detoxification, as a substrate for in GSH-xenobiotic adduct formation catalyzed by glutathione-S-transferase enzymes (GST).[19,20] GSH is synthesized by tandem reactions catalyzed by γ-glutamate cysteine ligase (GCL) and glutathione synthase (GSS). Several studies have already demonstrated that some carotenoids, such as β-carotene, lycopene, β-cryptoxanthin, and astaxanthin could increase GSH levels in different cell types. Mechanisms include the induction of GCL gene expression, activation of recycling GSH-dependent enzymes (e.g. GR), or regulation of GST activity.[21,22] Figure 1 depicts the main thiol-dependent reactions responsible for redox homeostasis in eukaryotic cells and the putative effects of dietary carotenoids on those.

The Nrf2-Keap1 system

Regulation of endogenous antioxidant responses is central to the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), through modification of redox sensitive thiol(-SH) groups of Kelch-like ECH-associated protein-1 (Keap1).[23] The mechanism relies on Nfr2-Keap1 protein-protein interactions, unless specific -SH residues of Keap1 are oxidized by electrophiles that are produced by oxidative metabolism [e.g., H_2O_2, nitro(so)lipids and α,β-unsaturated aldehydes, like 4-hydroxy-2-nonenal, produced by lipoperoxidation] or provided by dietary sources, like sulforaphane (in broccoli), curcumin (in *Curcuma longa*), and some (apo)carotenoids. These compounds are reactants for the Michael reactions that release Nrf2 in the cytosol for further phosphorylation and translocation to the nucleus (Figure 2). Keap1 does not only retain Nrf2 in the cytosol, but also promotes its ubiquitination and degradation in cells.[23] In the nucleus, phosphorylated Nrf2 binds to electrophile response elements (EpREs), also known as antioxidant responsive elements (AREs), which triggers the transcription of several cytoprotective genes, including those related to GSH and thioredoxin biosynthesis (e.g. GCL), enzymatic redox recycling, GST and other Phase II responsive enzymes. Cytoprotective Phase II responsive proteins include enzymes involved in the biotransformation of xenobiotics and drugs, such as NAD(P)H:quinone oxidoreductase 1 (NQO1), NRH:quinone oxidoreductase 2 (NQO2), heme-oxygenase 1 (HO-1), and the anti-apoptotic proteins Bcl-2 and Bcl-xL. More than 200 Nrf2-responsive genes were already identified upon activation by different electrophiles, like apocarotenoids, curcumin, and sulforaphane.[18]

Lycopene and other dietary carotenoids (including phytoene) were shown to induce the Nrf2-Keap1 system and gene transcription for the Phase II enzymes NQO1 and GCL (catalytic subunit) in MCF-7 (mammary cancer) and HepG2 (liver hepatocellular carcinoma) cells.[14] Structure/function studies demonstrated that an electrophilic α,β-unsaturated carbonyl group, a C=C bond conjugated to the -CHO aldehyde group, was necessary for the Michael reaction with Keap1, allowing Nrf2 release into the cytosol, and further Nrf2-mediated activation of EpRE.[24] It is now clear that this reactive aldehyde/ketone moiety is not part of the intact molecule of most carotenoids (excepting some xanthophylls, like astaxanthin), but rather, may exist as cleavage and oxidation derivatives of them.[24] Several apocarotenals and diapocarotenedials (products of BCO1 and BCO2 catalysis) were potential activators of the Keap1-Nrf2-EpRE system. Other potential reactive aldehydes can also be obtained from carotenoid enzymatic cleavage by the 9′,10′-monooxygenases.

Pro-inflammatory NF-κB/IκB pathway

Many dietary carotenoids also activate EpRE-responsive clusters through the NF-κB/IκB pathway. Briefly, NF-κB-dependent transcription is induced by various stimuli, including myokines and cytokines (e.g., tumor necrosis factor α, TNFα; IL-1β, etc.), oxidative stress, and UV radiation. The resultant NF-κB activation creates a positive proinflammatory condition, which triggers the activation of multiple immune cell responses. The NF-κB family is composed of five Rel homolog proteins, which primarily act as transcription factors: p65 (RelA), p50, p52, c-Rel, and RelB. Several apocarotenals,

like β-apo-10'-carotenal and lycopene-derivatives, directly interact with two key proteins of the NF-κB pathway: the IKKβ (leading to inhibition of its kinase activity) and the p65 subunit.[25] Additionally, new studies with ketocarotenoids, including astaxanthin, canthaxanthin, and (BCO1/BCO2)-cleavage derivatives, demonstrate their ability to elicit cross-talk in the Nrf2/NF-κB cascade responses.[25]

The 'Redox code'

Current studies on redox biology suggest that optimal redox balances in subcellular compartments are essential for cell survival, similarly as phosphorylation and pH control. Therefore, optimum ratios between pro-oxidants and antioxidants also exist for a given metabolic condition.[8] Numerous cellular processes, from gene expression up to post-translational modification of proteins and enzymes can be regulated by key signaling molecules, such as H_2O_2, $NO^{\bullet}$, and nitrosyl glutathione (GSNO), within subcellular and integrative redox circuits. The 'redox code' approaches the central theory for the redox organization of life. Along with the organism's inherent genetic code and epigenetic and histone codes to guide the use of genetic information, the 'redox code' adjusts the nucleic information set within energetic, metabolic, and environmental constraints of the organism.[8] These adjustments are performed at subcellular level and represent the spatial-temporal integration of $NAD(P)^+$/NADPH ratios derived from organelle metabolism and subsequent ROS/RNS production linked to biomolecules' oxidation.[8,19,20,26]

Intracellular redox balance directly responds to metabolic changes expressed in terms of $[NAD^+]$/[NADH] and $[NADP^+]$/[NADPH] ratios.[27] Upon excess energy-nutrient

supply, an elevated reductive condition is generally imposed on the cell by activation of the pentose phosphate pathway, synthesis of NADPH, and subsequent drop of the [$NADP^+$]/[NADPH] ratio, especially in the cytosolic compartment. Under these circumstances, higher levels of endogenous antioxidants are induced (e.g., GSH and thioredoxin), biosynthetic/anabolic processes are triggered, and energy-rich cells are metabolically prompted to undergo proliferation or differentiation processes, depending on other necessary inter- and intracellular factors.[20] In mitochondria, the link between the two nicotinamide-dependent redox switches requires the key enzyme nicotinamide nucleotide transhydrogenase (NNT), which is vital in free radical detoxification and redox balances.[19,20,26,27] However, subtoxic ROS/RNS concentrations lead to alterations in cellular and extracellular redox states, accelerating the formation of redox signaling molecules at levels sufficient to upregulate the expression of cytoprotective genes, and improving cellular (and compartmental) antioxidant defenses. Besides H_2O_2, $NO^{\bullet}$, and GSNO, some lipid peroxidation byproducts, such as 4-hydroxy-2-nonenal (an α,β-unsaturated aldehyde) and nitro-/oxylipids, also activate Nrf2-Keap1 and NF-κB pathways at some levels.[8,20] As the oxidative challenge increases in intracellular compartments, higher demands on reestablishing the redox balance towards an overall reductive condition will impose a different scenario, by switching on the transcription of redox-responsive genes that increase antioxidant defenses and turning off other genes that could aggravate oxidative conditions within cells (e.g. by overexpression of oxidases and cytochromes). The overall cellular redox switch (overall, because redox control is clearly site-specifically determined) could be set to define different phenotypic fates: (i) proliferation/differentiation (when reductive conditions prevail); (ii) a steady-state condition, G_0 (mostly sustained by adjustments in the

Keap1-Nrf2-EpRE system); (iii) inflammation-like patterns (modulated by NF-κB cascades); (iv) apoptosis (through the AP-1 pathway, if proper apoptotic machinery is available for that cell type); and (iv) necrosis (disseminated and uncontrolled oxidative damage).[8,19,20] Inevitably, due to variable cellular plasticity and redox responsiveness, different cell types have different oxidative tolerances or redox ranges for each of the phenotypic fates. For example, human hepatocytes have a higher capacity to proliferate and to cope with higher oxidative challenges than neurons or glia cells. Apoptosis activation also varies among different human cells.[28] Studies on mitochondria suggested that the intracellular superoxide ($O_2^{\bullet -}$) concentrations may directly drive the switch from apoptosis to necrosis, revealing an incremental effect on oxidative conditions associated with an intracellular ATP depletion.[29] The hypothetical redox switch in eukaryotic cells, and the manner of how different phenotypes are elicited by the redox switches in different plasticity/function cell are summarized in Figure 3 (detailed information in the legend).

In summary, the endogenous ROS/RNS production driven by essential cellular processes is normally counteracted by an intricated net of (compartmentalized) antioxidant systems, which respond, for proper adjustments, to many redox-active signaling molecules that trigger endogenous antioxidant defenses. Carotenoids and other anti-/prooxidants present in human diet will account for additional disturbances in such redox switches, either in an integrative perspective or in a subcellular mode of action. Astaxanthin, for example, has been investigate for a putative mitochondria-targeted activity in vivo.[16,30] The bioactive content in food stuff (carotenoid sources), availability, transport, metabolic modification, excretion, etc. will strongly influence their real effect *in situ*. The role of dietary carotenoids is particularly intriguing since: (i) carotenoids possess both anti- and pro-

oxidant properties, which is structure-related and dependent on local physicochemical conditions; (ii) (apo)carotenoids activate the Nrf2-Keap1 pathway, upregulate cellular antioxidant defenses and rebalance cellular redox conditions; (iii) chemical moieties in their structure also trigger pro-inflammatory NF-κB cascades, which could enhance the oxidative insult; and (iv) all the aforementioned properties are modulated by synergism with other dietary carotenoids (apocarotenoids?) and antioxidants, such as ascorbic acid (vitamin C) and α,γ-tocopherols and tocotrienols.[18-20]

Perspectives

Therefore, based on the multifaceted role of carotenoids in redox metabolism, it is difficult to define a single and beneficial dose of dietary carotenoids necessary to promote health in humans. The variety of biological targets (depending on the inflicting disease) and many nutrigenetic/nutrigenomic issues demonstrate that individually distinct physiological outcomes could be expected from a fixed (recommended) dose of common dietary carotenoids. More cohesive integration of "omics" information in cells and tissues from studies with carotenoid-fed healthy subjects and patients of specific diseases is necessary to shorten the gap between the carotenoid content in food stuff (towards recommendation intake values) and their physiological benefits for human health.

Abbreviations Used

AP-1, activator protein-1; AREs, antioxidant responsive elements; BCO1, 15,15′-oxygenase 1; BCO2, 9′,10′-oxygenase 2; Car-OO•, carotene-peroxyl radical; C/EBPs, CCAAT-enhancer binding proteins; CD36, cluster determinant 36; EpREs, electrophile promoter responsive elements; FDA, US Food and Drugs Administration; GCL, γ-glutamate-cysteine ligase; GR, glutathione reductase; GSH, reduced glutathione; GSNO, nitrosyl-glutathione; GSS, glutathione synthetase; GSSG, oxidized glutathione; GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; HO•, hydroxyl radical; HO-1, heme-oxygenase-1; IκB, inhibitory κB protein; IL-1β, interleukin-1β; Keap1, Kelch-like ECH-associated protein-1; LOOH, lipid hydroperoxides; NFκB, nuclear factor *kappa*-light-chain-enhancer of activated B cells; NNT, nicotinamide nucleotide transhydrogenase; NO•, nitric oxide; NQO1, NAD(P)H:quinone oxidoreductase 1; NPC1L1, Niemann-Pick C1-Like 1 transporter; NR, nuclear receptors; Nrf2, nuclear factor erythroid 2-related factor 2; $^1O_2$, singlet oxygen; PIDI, Prudent Individual Daily Intakes; pO_2, oxygen partial pressure; PPAR, peroxisome proliferator-activated receptors; RARs, retinoic acid receptors; RDA, Recommended Daily Allowance; RDI, Reference Daily Intake; RO•, alkoxyl radical; ROO•, peroxyl radical; ROS/RNS, reactive oxygen/nitrogen species; RXRs, retinoic X receptors; SR-BI, scavenger receptor-class B type I; TNFα, tumor necrosis factor α; UV, ultra violet radiation; WHO, World Health Organization.

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Figure Captions

Figure 1. Integration of glycolysis, the pentose phosphate pathway (PPP), biosynthesis of cholesterol and fatty acids (FA) with the thiol(-SH) based antioxidant system via the $NADP^+$/NADPH ratio. At the left side, bottom, the dashed boxes summarize papers showing that: (i) lycopene, β-carotene, β-cryptoxanthin, and/or astaxanthin treatment increased GSH content in different cells;[21,22] (ii) suggestive activation of the detoxifying enzyme GST by lycopene, β-carotene, β-cryptoxanthin, and astaxanthin in hepatocytes; and (iii) inhibition of GST activity in hepatic tissues by the apocarotenoid crocetin (reference linked). The figure does not consider intracellular compartmentalization, as some of the reactions primarily occur in the mitochondrial matrix (e.g. mediated by Trx2 and Grx2), whereas other prevail in cytosol, such as GPX-catalyzed reactions. Abbreviations: FA, fatty acid; GPX, glutathione peroxidase; GR, glutathione reductase; Grx2, cytosolic/mitochondrial glutaredoxin; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; LOH, lipid alcohol; LOOH, lipid peroxide; PPP, pentose phosphate pathway; Prdx3, mitochondrial peroxiredoxin; Trx2, mitochondrial thioredoxin; TrxR, cytosolic Trx-reductase; TrxR2, mitochondrial Trx-reductase.

Figure 2. Electrophile activation of Nrf2-Keap1 system by: (1) Reactive Oxygen/Nitrogen Species (ROS/RNS), or (2) Carotenoids and Apocarotenoids. Specific thiol(-SH) groups in Keap1 are sensitive to oxidation by ROS/RNS or to electrophiles, such as dietary carotenoids and apocarotenoids. Upon direct oxidation or Michael`s adduct formation, Nrf2 escapes Keap1 assisted ubiquitinylation (and further degradation) and, after PKCδ-

catalyzed phosphorylation (at Ser_{40}), phosphorylated Nrf2 migrates to the nucleus. Electrophiles, lipid hydroperoxide (LOOH), and/or hydrogen peroxide (H_2O_2) activate PKCδ via Src proteins. In the nucleus, phosphorylated Nrf2 associates with other transcription factors, such as CBP/p300 and Maf proteins, forming a transcription complex that binds at Electrophile-promoter Responsive Elements (EpREs) region of several cytoprotective genes, including heme-oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), and superoxide dismutase (SOD). The transcription complex is dismantled by further Fyn-catalyzed phosphorylation of Nrf2 at Tyr_{568}, and by translocation to the cytosol for degradation.

Figure 3. (A) The hypothetical $NAD(P)^+$/NAD(P)H-responsive redox switch of eukaryotic cells that triggers distinct phenotypic fates depending on cellular redox balance. From a basal condition (optimum redox balance), the redox switch elicit inflammatory pathways, apoptosis, or necrosis following increasing oxidative conditions, whereas unclear "reductive stress" mechanisms are triggered when NAD(P)H coenzymes prevail in cellular compartments. There is evidence of AKT/mTOR and AMPK cascades' activation under reductive conditions. An increase of the cellular antioxidant capacity (from diet intake or generated endogenously) slides the antioxidant "seesaw" pivot point to the right, attenuating the magnitude of ROS/RNS production in cell. However, an excessive antioxidant load in cells (sliding further to the right) could prevent beneficial processes mediated by the Nrf2-Keap1-EpRE system. Adapted from: Niess, A.M.; Simon, P. Response and adaptation of skeletal muscle to exercise--the role of reactive oxygen species. *Front Biosci.* **2007**,*12*, 4826-4838. (B) Carotenoids and apocarotenoids in bloodstream

could trigger distinct transcriptomic, proteomic/redoxomic, and metabolomic patterns in different cell types of the same subject, based on the diversity of redox switches for different cell functions and morphologies. At the left side, bottom, the color scale (+3.0 to -3.0) illustrates the effects of lycopene on the expression of some genes in MCF-7 breast cancer cells: (i) upregulation of GGADD45A (apoptosis), CDC25A (cell cycle regulation), RARA and RXRR (receptors), MSH2 and DDB2 (DNA repair); (ii) downregulation of CASP8 (apoptosis), CDNE (cell cycle regulation), AR (receptors), and MRE11A (DNA repair).[14]

Table 1. Daily Carotenoid Intake in Brazil, Spain, and USA, Based on National Surveys.

		Carotenoid (mg/day)[a]					
Country	Statistics	αCAR	βCAR	βCRY	LUT+ZEA	LYC	TOTAL
Brazil[b]	n = 34003 2008-2009 (♂♀, ≥10 y)	0.16	1.47	0.11	1.15	1.44	4.32
Spain[c]	n = 3000 2009-2010 (♂♀, ≥18 y)	0.27	1.46	0.32	1.24	3.06	6.36[f]
USA[d]	n = 9042 2009-2010 (♂♀, ≥02 y)	0.38	1.94	0.08	1.36	5.26	9.02[f]
PIDI[e]		N.A.	5.2 – 6.0[e]	N.A.	N.A.	N.A.	9.0 – 18.0[e]

[a] Abbreviations: αCAR, α-carotene; βCAR, β-carotene; βCRY, β-cryptoxanthin; LUT+ZEA, combined lutein and zeaxanthin intakes; and LYC, lycopene.

[b] data from: Sartori, A.G.O.; Silva, M.V. Main food sources of carotenoids, according to the purpose and degree of processing, for beneficiaries of the 'Bolsa Família' in Brazil. *Food Sci. Technol. (Campinas)*, **2014**, *34*, 408-415. http://dx.doi.org/10.1590/fst.2014.0056.

[c] data from: *(c1)* Estevez-Santiago, R.; Beltran-de-Miguel, B.; Olmedilla-Alonso, B. Assessment of dietary lutein, zeaxanthin and lycopene intakes and sources in the Spanish survey of dietary intake (2009-2010). *Int. J. Food Sci. Nutr.*, **2016**, *67*, 305-313. *(c2)* Beltran-de-Miguel, B.; Estevez-Santiago, R.; Olmedilla-Alonso, B. Assessment of dietary vitamin A intake (retinol, alpha-carotene, beta-carotene, beta-cryptoxanthin) and its sources in the National Survey of Dietary Intake in Spain (2009-2010). *Int. J. Food Sci. Nutr.*, **2015**, *66*, 706-712.

[d] data from: NHANES, What We Eat in America (2009-2010). In: Nutrient Intakes from Food: Mean Amounts Consumed per Individual, by Gender and Age. U.S. Department of Agriculture, Agricultural Research Service, **2012**.
Available: https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/0910/tables_1-40_2009-2010.pdf (access on May 11, 2018)

[e] data from: Institute of Medicine. **2000**. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: The National Academies Press. https://doi.org/10.17226/9810 (access on May 11, 2018). Abbreviation: N.A., not available.

[f] calculated as a sum of carotenoid contents shown in Table 1.

Figure 1

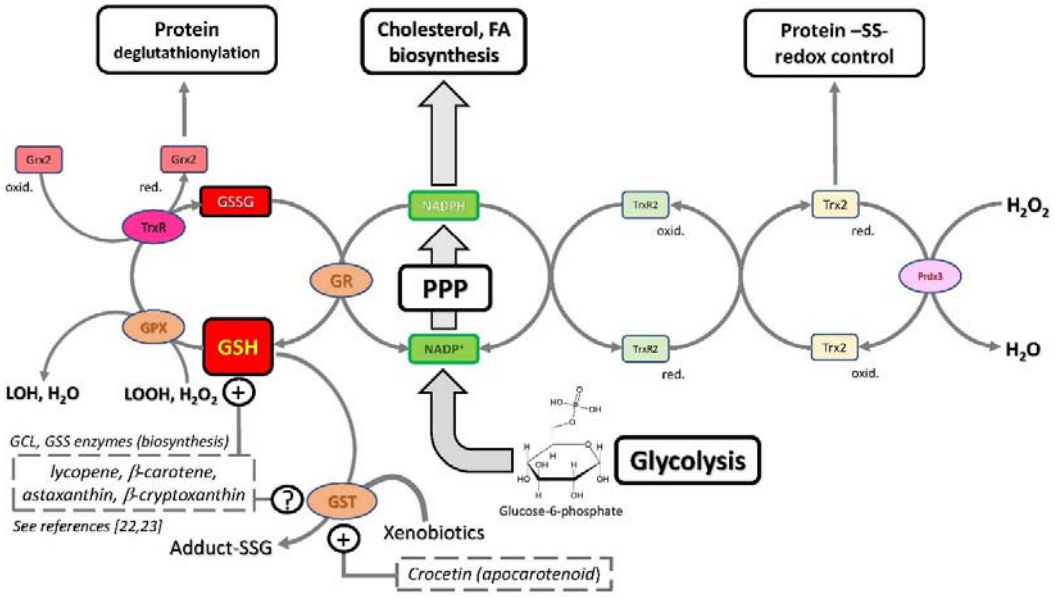

Protein deglutathionylation
Cholesterol, FA biosynthesis
Protein –SS- redox control
Grx2
oxid.
Grx2
red.
GSSG
TrxR
NADPH
TrxR2
oxid.
Trx2
red.
H_2O_2
GR
PPP
Prdx3
GPX
GSH
NADP+
TrxR2
red.
Trx2
oxid.
H_2O
LOH, H_2O
LOOH, H_2O_2
GCL, GSS enzymes (biosynthesis)
lycopene, ß-carotene, astaxanthin, ß-cryptoxanthin
See references [22,23]
GST
Adduct-SSG
Xenobiotics
Crocetin (apocarotenoid)
Glycolysis
Glucose-6-phosphate

Figure 2

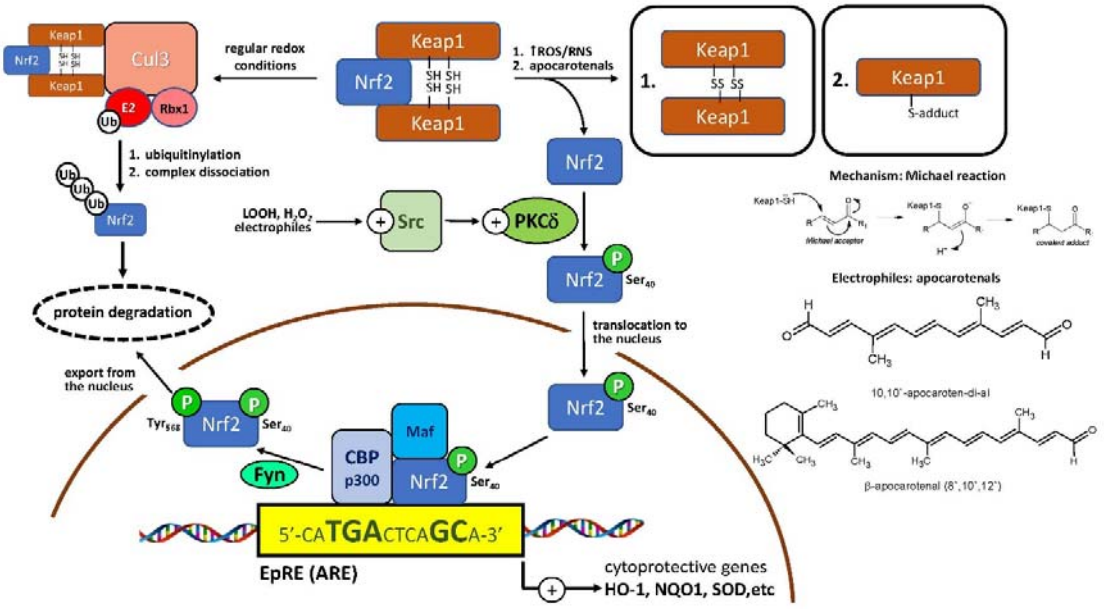
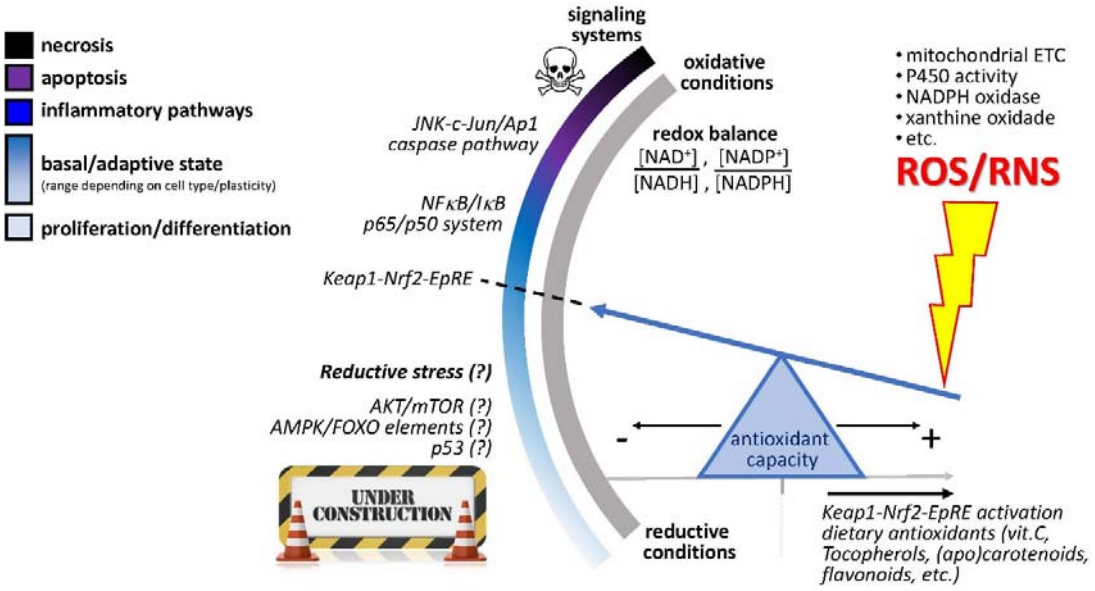
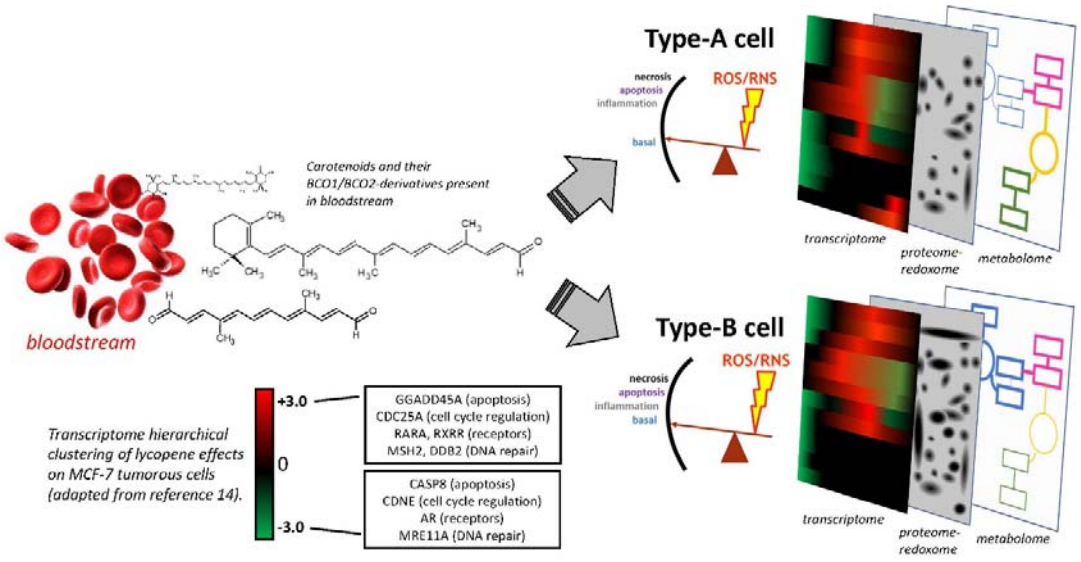

Keap1
Nrf2
Cul3
E2
Rbx1
Ub
regular redox conditions
1. ubiquitinylation
2. complex dissociation
protein degradation
LOOH, H2O2 electrophiles
Src
PKCδ
1. ↑ROS/RNS
2. apocarotenals
1.
2.
S-adduct
Mechanism: Michael reaction
Electrophiles: apocarotenals
10,10'-apocaroten-dl-al
β-apocarotenal (8',10',12')
translocation to the nucleus
export from the nucleus
Maf
CBP
p300
Fyn
5'-CATGACTCAGCA-3'
EpRE (ARE)
cytoprotective genes
HO-1, NQO1, SOD,etc

Figure 3

(A)


necrosis
apoptosis
inflammatory pathways
basal/adaptive state
(range depending on cell type/plasticity)
proliferation/differentiation
signaling systems
oxidative conditions
JNK-c-Jun/Ap1 caspase pathway
redox balance
[NAD+] , [NADP+]
[NADH] , [NADPH]
NFκB/IκB p65/p50 system
Keap1-Nrf2-EpRE
• mitochondrial ETC
• P450 activity
• NADPH oxidase
• xanthine oxidade
• etc.
ROS/RNS
Reductive stress (?)
AKT/mTOR (?)
AMPK/FOXO elements (?)
p53 (?)
UNDER CONSTRUCTION
antioxidant capacity
reductive conditions
Keap1-Nrf2-EpRE activation
dietary antioxidants (vit.C, Tocopherols, (apo)carotenoids, flavonoids, etc.)

(B)


Type-A cell
necrosis
apoptosis
inflammation
basal
ROS/RNS
transcriptome
proteome-redoxome
metabolome
Carotenoids and their BCO1/BCO2-derivatives present in bloodstream
bloodstream
Type-B cell
Transcriptome hierarchical clustering of lycopene effects on MCF-7 tumorous cells (adapted from reference 14).
+3.0
0
-3.0
GGADD45A (apoptosis)
CDC25A (cell cycle regulation)
RARA, RXRR (receptors)
MSH2, DDB2 (DNA repair)
CASP8 (apoptosis)
CDNE (cell cycle regulation)
AR (receptors)
MRE11A (DNA repair)

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