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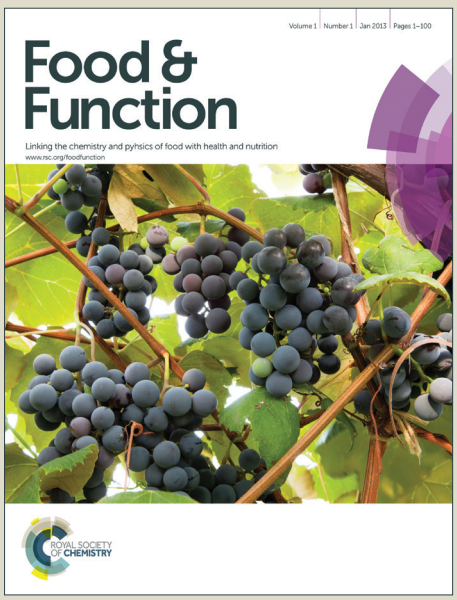

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A short-cut to inclusive biological actions of dietary polyphenols: modulation of the nitrate:nitrite:nitric oxide pathway in the gut

Bárbara S Rocha, Carla Nunes, Cassilda Pereira, Rui M Barbosa, João Laranjinha

Faculty of Pharmacy and Center for Neurosciences and Cell Biology, University of Coimbra, Health Sciences Campus. Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

Corresponding author

João Laranjinha
Faculty of Pharmacy and Center for Neurosciences and Cell Biology,
University of Coimbra,
Health Sciences Campus,
Azinhaga de Santa Comba,
3000-548 Coimbra, Portugal
Phone: +351-239488466
Email: laranjin@ci.uc.pt

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Abstract

Dietary polyphenols are complex, natural compounds with recognized health benefits. Initially attractive to the biomedical area due to their in vitro antioxidant properties, the biological implications of polyphenols are now known to be far from the acute ability to scavenge free radicals but rather to modulate redox signaling pathways. Actually, it is now recognized that dietary polyphenols are extensively metabolized *in vivo* and that the chemical, biophysical and biological properties of their metabolites are, in most cases, quite different from the ones of the parent molecules. Hence, the study of the metabolic,

absorptive and signaling pathways of both phenolics and derivatives has become a major issue. In this paper we propose a short-cut for the systemic effects of polyphenols in connection to nitric oxide (•NO) biology. This free radical is an ubiquitous signaling molecule with pivotal functions *in vivo*. It is produced through an enzymatic pathway but also through the reduction of dietary nitrate and nitrite in the human stomach. At acidic gastric pH, dietary polyphenols, in the form they are conveyed in foods and at high concentration, promote nitrite reduction to •NO but also embark in a complex network of chemical reactions to produce higher nitrogen oxides with signaling functions, namely by inducing post-translational modifications. Modified endogenous molecules, such as nitrated proteins and lipids, acquire important physiological functions. Thus, local and systemic effects of •NO such as modulation of vascular tone, mucus production in the gut and protection against ischemia-reperfusion injury are, in this sense, triggered by dietary polyphenols. Evidences to support the signaling and biological effects of polyphenols by modulation of the nitrate-nitrite-NO pathway will be herein provided and discussed. General actions of polyphenols encompassing the absorption and metabolism in intestine/liver are short-cut via the production of diffusible species in the stomach that have not only a local but also a general impact.

The *in vivo* beneficial effects of dietary polyphenols have been largely studied in connection with their bioavailability which incorporates a sequence of steps intrinsically related to the biological compartments that, after consumption in the diet, the alimentary bolus travels through. This implies that even before enterocyte-dependent metabolization, occurring during absorption in the gut, as soon as a polyphenol-rich food enters the oral cavity it starts to suffer interactions with other molecules and structural modification.[1] Accordingly, in addition to modifications during and after absorption, including conjugation to glucuronides, sulphate and methyl groups, major chemical modifications occur in the small intestine, determining in most cases the absorption of metabolites with chemical and biological properties rather different from the ones of the parent molecules.[2] In the colon, given the

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huge amount of bacteria (estimates of 10^{11} bacteria/g are being consistently reported),[3] the polyphenols that survived all the way through the gut, or that were chemically modified but not absorbed upstream, are now at the mercy of the microbiota and several metabolites (such as phenolic acids) with still unrecognized biological impact may be produced.[4]

In summary, the current view for the inclusive effects of dietary polyphenols considers their bioavailability and the potential effects of metabolites derived from the original structures consumed in the diet. Such a pathway, depicted in figure 1, consists in a long and winding road but, in fact, it has been almost exclusively the theoretical background for the recent research on polyphenols since the supposed acute antioxidant activity *in vivo* has been largely abandoned due to lack of evidence.[5, 6] These are important research lines but the identification of polyphenol metabolites, their absorption rates and ensued signaling effects remains a herculean challenge. Moreover, with the recent reports on the outstanding diversity of the gut microbiota and the impact that these microorganisms have on the modification of virtually almost all nutrients,[7] the question comes as to whether the same food is differently metabolized by two individuals with different microbiome profiles.

Still, the health benefits of polyphenols are epidemiologically robust.[8, 9] Here, we focus on a hitherto undervalued pathway for systemic biological effects of dietary polyphenols in connection to nitric oxide (•NO) biology that short-cuts the standard pathway above described (figure 1). The production of such ubiquitous messenger in the human stomach (•NO) may actually be highly dependent on the simultaneous consumption of polyphenols and nitrate.[10, 11] Moreover, the reaction of polyphenols with nitrite (originating from nitrate reduction) yields, in addition to •NO, nitrated and nitrosated derivatives endowed with biological activity that goes far beyond the stomach mucus and mucosa.

We hereby provide evidence supporting the notion that the systemic effects of unmodified dietary polyphenols (before modification and absorption in the intestine) may be conveyed by •NO and related nitrogen oxides produced upon nitrite reduction by polyphenols in the stomach. Hence, the nitrate-nitrite-NO pathway provides the unique conditions for dietary polyphenols to act in accordance with the physicochemical properties of its unmodified forms (aglycons) independently of its absorption and metabolization in the gut.

1. The nitrate-nitrite-NO pathway

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The last decade witnessed an increased interest in the health-promoting effects of dietary nitrate. Since the 1990's, when Lundberg[12] and Benjamin[13] described the chemical production of •NO from nitrate in the stomach, previous work from the 1970's gained a renewed interest on the context of the now-called *nitrate-nitrite-NO pathway*.

Actually, nitrate, from green leafy veggies and roots, is known for many years to undergo an enterosalivary circulation *in vivo*,[14] thereby increasing salivary nitrite.[15, 16] By doing so, the human body rescues about 25% of nitrate ingested upon a regular meal, from the systemic circulation into the oral cavity. Nitrate is then reduced to nitrite by bacteria harbored in the posterior tongue clefts[17] and, as recently realized, reaches the stomach boosting •NO production, with both local and systemic effects.[11, 18] These include mucosal vasodilation, mucus production and modulation of redox reactions with ensued biological impact (reviewed in [19]). Some of these effects will be address below with more detail. However, most nitrite escapes gastric acid and is absorbed in the small intestine.[20] As soon as 30 minutes after a nitrate load, there is an increase in both plasma nitrate (*c.a.* 430 μM) and nitrite (*c.a.* 230 nM).[20] In fact, at the expenses of the enterosalivary circulation of nitrate, the systemic concentrations of both anions remain high for 5-6 hours, affording a constant delivery of nitrite to the stomach, keeping pace of •NO generation.[20] The plasma nitrite concentration is now known to be of great physiological relevance as evidence support that nitrite can be reduced to •NO in the tissues (for a detailed review see [21]) by different enzymatic systems. These include xanthine oxidase,[22] hemoglobin, myoglobin[23] and mitochondrial enzymes,[24-26] to cite just a few. The ability of these proteins to reduce nitrite is enhanced under hypoxic conditions, when conformational changes drive nitrite into the heme moiety, thereby producing •NO and the oxidized form of the protein.[27] The univalent reduction of nitrite guarantees •NO bioavailability precisely when the L-arginine-NO pathway catalyzed by the family of nitric oxide synthases (NOS) is inhibited. This is because NOS require O_2 as a co-factor for •NO synthesis; thus, during ischemia, such enzymatic pathway becomes shut down.[28] Intriguingly, Vanin and coworkers showed that under low pO_2, endothelial NOS (eNOS) acquires a *nitrite-reductase activity*, contributing also for nitrite reduction.[29] Hence, nitrite may be regarded as a systemic reservoir of •NO ensuring its bioactivity when the classical enzymatic pathway (NOS) is hindered. For this reason, much attention has been given on nitrite signaling during hypoxia and important protective functions have been already described, ranging from protection against ischemia-reperfusion injury,[30] reduction of infarct size[31] and prevention of age-related vascular dysfunction.[32]

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A challenging issue on the biology of nitrite is the critical role of the diet as the trigger and ultimate modulator of endogenous •NO signaling. Moreover, given the complexity of the human diet, different reactions between foods and endogenous macromolecules (such as polyphenols and nitrate) may significantly influence pathophysiological events not only in the gut but also systemically.

2. Overview of the pathways and mechanisms underlying the biological impact of polyphenols

Epidemiologically, it is widely accepted that the regular consumption of vegetables promotes good health. Polyphenols have been associated with this hypothesis due to the observation that most oligomers (e.g., procyanidins) and glycosides (the original forms in plants) are antioxidants *in vitro*.[5] In a seminal paper from 1990, Bors et al determined the rate constants of flavonoid interaction with selectively generated free radicals by pulse radiolysis (as well as the stability of the "antioxidant-derived radical") and established the structural principles for effective radical scavenging by flavonoids;[33] such principles are (a) the *o*-dihydroxy (catechol) structure in the B ring, (b) the 2,3-double bond in conjugation with a 4-oxo function and, finally, (c) additional presence of both 3- and 5-hydroxyl groups for maximal radical scavenging activity. However, the chemical details supporting the effective scavenging activity (notably the catechol structure) are modified in the gut during absorption and, moreover, most of these compounds are poorly absorbed *in vivo* (about 90% reach the colon unmodified).[34] Other polyphenols are extensively metabolized in the gut limiting their ability to donate a hydrogen atom and therefore to act as acute free radical scavengers.[5, 35] This implies that the plasma concentration of most of the original structures consumed in the diet and proven to be antioxidants *in vitro* is extremely low and can hardly add to potential antioxidant effects supported by endogenous molecules such as alfa-tocopherol and ascorbate, among other compounds.[36]

Additionally, the notion of a "systemic antioxidant" can be hardly supported on basis of the redefinition of oxidative stress as a disruption of redox signaling pathways, emphasizing discreet and compartmentalized cellular redox circuits[37].

Taken together, these observations raise serious questions on the efficacy of the acute antioxidant action of polyphenols *in vivo*. Yet, numerous studies are still being published reinforcing the health-promoting properties of polyphenols. Just to mention a few related with vascular function, functional studies, show that dietary polyphenols improve flow-

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mediated vasodilation (FMD) in humans upon transient ischemia,[38] attenuate the risk factors of metabolic syndrome[9] and improve endothelial function and •NO status in healthy volunteers.[8] The lack of congruency on the outcome of polyphenol consumption for human health and the acute antioxidant activity led to *a change of paradigm in polyphenol research.*

It is becoming evident that many of the effects of polyphenols rely on the modulation of signaling pathways, by interacting with redox-sensitive enzymes and receptors as well as by activating transcription factors (reviewed in [39, 40]). Just to give a few interesting examples, flavonoids may fit in the ATP-binding site of protein kinases, affecting the phosphorylation state of downstream targets; this specific reaction has actually been demonstrated to inhibit the cell cycle.[41] In addition, quercetin may be metabolized to hydrophilic aglycones that are translocated into the active site of key enzymes involved in the production of reactive nitrogen oxides, such as xanthine oxidase and lipoxygenase [42, 43]. This process is favored under oxidative stress, such as inflammation, which pinpoints this mechanism as a strategy to counteract cardiovascular diseases.[44] Moreover, polyphenols have also been shown to inhibit 5-lipoxigenase and NADPH oxidase in the vasculature, again with putative anti-inflammatory properties.[45-47] It is of note that the action upon these enzymatic activities may encompass an indirect antioxidant activity. Interestingly, most of these effects are observed for some of the metabolites but not for the original molecules.[47] Indeed, the recognition that polyphenol metabolites are not only bioactive but also have biological implications rather different from the original molecules, prompted intensive studies on the metabolism of polyphenols in the gut.

An important concept that has recently emerged is that the redox regulation of cell functions by polyphenols also entails the activation of a stress cell response. Paradoxically, polyphenols may be beneficial for human health because they can act as toxins, promoting increased expression of cellular defense enzymes via the KeapI-Nrf2-ARE pathway,[48] a process that one could describe as “phytohomersis”.

However, the discussion pertaining the *in vivo* “antioxidant action” of dietary polyphenols cannot exclude the capacity of polyphenols to establish H-bonds and hydrophobic interactions, supporting its binding to membranes[49, 50] where they (overcoming their isotropic dilution), may achieve local concentrations (at membrane-water interfaces) high enough to act as antioxidants by recycling α-tocopherol on a structure-dependent way.[51, 52] In fact, in 1994 we have proposed that phenolic compounds could regenerate vitamin E at the low

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density lipoprotein surface via a recycling mechanism, thus acting as co-antioxidants.[53]

In recent years, a flourishing research field is focused on describing polyphenol's modification all the way from ingestion to absorption or excretion. Thus, not only host-dependent biochemical modifications have been reported (oxidation, sulfation, glucuronidadion)[54] but also endogenous bacteria seem to play an important role on polyphenol decomposition.[55, 56] The human microbiota may extensively metabolize dietary polyphenols.[57, 58] In fact, the amount and diversity of bacteria increases from the proximal towards the distal gut[3] and therefore, these modifications are likely to occur mostly in the small intestine and the colon.[59] The way gut bugs transform polyphenols is actually quite interesting because although humans share a core microbiota, there are variations due to genetic background, environmental and dietary lifestyles. Therefore, is likely that the same polyphenol, ingested by individuals with different microbiome profiles, may be differently metabolized, thereby producing diverse metabolites with putative distinct biological impact.

Taken these considerations altogether, it is becoming widely accepted that the bridge between diet, polyphenols, health and disease may be mechanistically supported by the modulation of redox signaling pathways, modification of gene expression and of enzymatic activities. The health benefits of polyphenols rely in a rather complex network of events (metabolization, absorption, gut and systemic chemical interactions) each of them highly vulnerable to a wide range of individual characteristics (microbiota profile, genetics, dietary behavior). However, we want to stress that, conversely to the situation after absorption, unmodified polyphenols reach the stomach in high concentrations and, therefore, the thermodynamic and kinetic properties that support their antioxidant activity *in vitro* may also apply *in vivo* in the gastric compartment. By this way, the biochemistry of polyphenols in the stomach and intestine, in connection to the nitrate-nitrite-NO pathway, may constitute a short-cut for the biological effects of these molecules, justifying their impact on human health.

3. The interaction of polyphenols with nitrite in the stomach: from local to systemic biological impact

The recent advances on the biology of polyphenols have shed light on critical aspects involving metabolization, absorption, and modulation of local and systemic signaling pathways (reviewed in [6]). It is now known that as soon as polyphenol-rich foods are ingested, a multitude of complex interactions take place. In the oral cavity, proline-rich

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proteins form complexes with dietary tannins, preventing their downstream absorption in the gut.[1] Although such interactions in the upper gastrointestinal (GI) tract prevent the absorption of large flavanols, they are not devoid of biological significance. For instance, procyanidins interact with the cell membrane of enterocytes, stabilizing lipid rafts and ensuring the integrity of cellular biochemical pathways, namely by mitigating the extracellular signal-regulated kinases (ERK) signaling cascade.[60] Still in the intestine, dietary polyphenols (from a red wine extract) seem to protect the mucosa by interfering with different signaling inflammatory cascades.[61] Using an enterocyte cell model, we provided support that red wine polyphenols inhibited the production of pro-inflammatory mediators (IL-8 and •NO) and the expression enzymes such as iNOS and COX-2 in a dose-dependent manner. Both events were associated with the suppression the NF-κB pathway. Moreover, phenolic red wine extract was also shown to inhibit protein tyrosine nitration, a biomarker of nitrosative stress, induced by a mixture of cytokines, most likely due to the suppression of iNOS and thus •NO overproduction.[61] Globally, this study suggests that red wine polyphenols are able to ameliorate intestinal inflammation.

Nonetheless, before reaching the intestine, the reductant properties of polyphenols cannot be disregarded in the stomach. Upon a normal serving comprising a source of polyphenols (almost ubiquitous in the human diet) and nitrate, the stomach provides an exquisite environment for the production of •NO. Thermodynamically, polyphenol radicals present a reduction potential (E) of roughly 0.35-0.7 V, while nitrite is 0.8 V, which favors the univalent reduction of nitrite to •NO. This reaction was proposed years ago[62] and, accordingly, we have demonstrated that under simulated gastric conditions, caffeic acid is oxidized to the o-semiquinone radical while nitrite is reduced to •NO.[11] This observation also applies to other polyphenols, such as chlorogenic acid[10] but only recently we were able to establish the proof of concept that the ingestion of polyphenol- and nitrate-rich foods boosts •NO production in the human stomach.[18] Although the rate constants for nitrite reduction by polyphenols under these conditions have yet to be determined, the high concentration of both reagents (nitrite and polyphenols) attained in the stomach would kinetically favor the reaction. These observations establish a direct link between polyphenols and •NO biology. Thus, the gastroprotective effects of •NO[63-65] may be directly modulated by the diet, depending on whether polyphenols are readily available for nitrite reduction. Several other conditions rather than the acidic pH, promote the interaction of polyphenols and nitrite in the stomach. The high pO_2 and pCO_2 in the gastric headspace, the presence of HCO_3^- in the gastric juice, the activity of NOX enzymes and the peristaltic movements of the stomach

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itself, promote a somehow complex network of chemical reactions (reviewed in [66, 67]). Indeed, several nitrogen oxides (radicals or not) may be produced under such conditions [68]. Some of them, dinitrogen trioxide (N_2O_3), nitrogen dioxide radical ($^{\bullet}NO_2$) and peroxynitrous acid (ONOOH) may induce post-translational modifications in both endogenous and exogenous macromolecules (most notably lipids and proteins). In accordance, red wine poses as a privileged dietary product for most of these reactions. In one hand, promotes nitrite reduction to $^{\bullet}NO$ in the human stomach due to the remarkable concentration of polyphenols[11, 18] and, on the other hand, the ethanolic fraction undergoes O-nitrosation reactions, yielding ethyl nitrite, likely induced by HNO_2 and/or N_2O_3.[69, 70] Interestingly, ethyl nitrite is a powerful vasoactive compound *via* $^{\bullet}NO$ release, affording an unexpected physiological relevance to this compound if absorption into the blood stream, where further $^{\bullet}NO$ release is prone to occur. Although this is an attractive hypothesis, it is yet to be confirmed. Still, evidences on nitrite-dependent nitrosation in the stomach are continuously emerging and unpublished results from our group indicate that cysteine-rich glycoproteins are S- and N-nitrosated upon exposure to nitrite under gastric conditions. This post-translational modification was detected in the gastric mucus and mucosa and red wine polyphenols were shown to inhibit protein nitrosation, suggesting competitive reactions between the phenoxyl radicals and nitrite-derived oxides.

The biochemical scenario for the gastric effects of polyphenols via redox interaction with dietary nitrite has recently been updated to include the modulation of nitrite-dependent nitration of both proteins and lipids. Recently, dietary nitrate, through the production of nitrite, has been shown to induce pepsin nitration and inactivation.[71] By using an *in vivo* model of secretagogue ulcers, we have shown that nitrite was able to induce pepsin nitration at acidic pH. Moreover, the same biochemical modification was observed when human saliva, obtained upon the ingestion of lettuce (nitrate load), was instilled in the rat stomach. This observation affords physiological relevance to nitrite-dependent nitration as nitrite, generated endogenously and in the presence of salivary inhibitors of protein nitration (e.g., thiocyanate, urate), is still able to induce pepsin nitration.[71] Moreover, *in vitro* results pointing to a decrease of the proteolytic activity of pepsin upon nitration, were translated *in vivo* into an diminished erosion of the gastric mucosa, thereby preventing the development of gastric ulcers.[71] Both pepsin nitration and amelioration of gastric ulcers were prevented when exogenous urate was administered, suggesting that $^{\bullet}NO_2$ may be the *ultimate* nitrating agent arising from nitrite at acidic pH (unpublished observations). Similarly, conjugated linolenic acid (CLA) is also a target for nitrite-dependent nitration in the

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stomach;[72] Bonacci and co-workers showed that the nitroalkene is absorbed systemically and may act as an anti-inflammatory in different body systems.[72, 73] Polyphenols have not only the ability to interact with nitrite to boost •NO production, but can also actively participate as an intermediary in these reactions. Actually, some polyphenols may undergo competitive nitration and be themselves nitrated.[74] This is an issue that clearly needs to be addressed in the context of the recently described modifications with physiological impact.

The biochemistry resulting from the interaction of polyphenols with nitrite in the stomach anticipates more general effects than the local impact summarized above. Although a direct role of nitrite or of •NO derived from nitrite reduction in the tissues has to be equated, it is also reasonable to consider that •NO generated in the stomach in a polyphenol-dependent reduction of nitrite can convey such systemic and beneficial effects (figure 2). This notion may be supported by several observations:

1) In an *ex vivo* model of the stomach, •NO generated from nitrite in the acidic lumen, can diffuse the gastric mucosa and be detected within the serosa, implying that *in vivo* •NO may reach the vasculature and modulate blood flow.[75] Importantly, when red wine is present in the gastric milieu, the steady state concentration of •NO increases up to threefold and the percentage of •NO diffused is *c.a.* 20% (whereas in the absence of red wine is *c.a.* 8%).[75] The increase of vascular tone is an important gastroprotective mechanism and these study supports that dietary polyphenols, in their unmodified form, may actively contribute to the protection of the gastric mucosa.

2) Locally modified proteins, ethanol and lipids may transduce •NO signaling, providing that these derivatives diffuse to inner layers of the organ and are absorbed into the systemic circulation, thus impacting on cardiovascular performance. For instance, by increasing gastric •NO, polyphenols may promote nitrosation and nitration reactions, yielding nitroso derivatives (nitrosothiols, ethyl nitrite) that may release •NO in the circulation. On the other hand, nitrated lipids formed in the stomach are known to exhibit anti-inflammatory properties in the vasculature (following absorption) with ensued vasoactive properties[70].

3) The increase of •NO in the stomach and (likely) •NO donors in the circulation (nitrosothiols, ethyl nitrite, nitrated lipids), may contribute to the raise of plasma nitrite.

4) Dietary supplementation studies with nitrate are connected with functional outcomes that have been typical assigned to •NO, such as amelioration of vascular function, protection against ischemia-reperfusion injury, improvement of insulin signaling in type 2 diabetes and decrease blood pressure among many other critical physiological pathways.[31, 76, 77] These studies, reporting several physiological responses to dietary nitrite, also afford biological

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significance to dietary polyphenols. For instance, the intake of a glass of beetroot juice was shown to decrease blood pressure and inhibit platelet aggregation in human volunteers [78]. However, in addition to nitrate, beetroot is also particularly rich in polyphenols (such as 4-hydroxybenzoic acid, caffeic acid, catechin, epicatechin and chlorogenic acid),[79] that may justify an intense gastric production of •NO with ensued vascular effects. Accordingly, our group has shown that the sole consumption of lettuce increases •NO in the human stomach in a way that cannot be simply assigned to nitrite reduction at acidic pH.[18] If it is true that lettuce is an important source of nitrate, it as also noteworthy that phenolics such as caffeic acid, luteolin and quercetin are also present[80] and may contribute to •NO generation despite the dramatic increase observed when phenolic- but not nitrate-rich foods were consumed afterwards.[18] In essence, the inclusive biological effects attributed to the nitrate-nitrite-NO pathway may be modulated by dietary polyphenols, given their chemical interactions in the stomach. However, such interactions may be more complex than anticipated and lead to unexpected outcomes *in vivo*. Recently, it was shown that a diet rich in both polyphenols (quercetin from apples) and nitrate (spinach), does not synergistically improve endothelial function or vascular tone.[8] Quercetin is a potent nitrite reductant at acidic pH and therefore diverting most nitrite to •NO production rather from intestinal absorption. This major gastric pathway may decrease the bioavailability of plasma and tissue nitrite and justify the timid vascular effects. Additionally, depending on their structure, the polyphenols are also targets for •NO-derived species. The doubt persists as to whether other phenolics, with a lower capability to reduce nitrite, could have similar results. Thus, more *in vivo* studies are required to clarify the synergistic effects of nitrate and polyphenols systemically.

5) Finally, a potential reduction of nitrite in the circulation by polyphenolic structures cannot be completely disregarded.

Steffen and colleagues have introduced the notion that mono-O-methylated flavanols and other flavonoids may interfere with •NO bioavailability.[47] In fact, these particular polyphenols, by inhibiting the superoxide radical-producing endothelial NADPH oxidase, may spare •NO from participating in a reaction with superoxide radical. We propose an additional pathway for the actions of polyphenols *via* interference with •NO metabolism: polyphenols in general are endowed with the redox properties to reduce nitrite in the stomach thus increasing •NO production which, in turn, may impact locally and systemically. Although this pathway has remained largely underappreciated, it can provide mechanistic

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insights that support most of the beneficial effects of polyphenols that do not fit in the concept of acute antioxidants.

4. Conclusions

Neither the effects of nitrate-derived •NO and related nitrogen oxides are new, nor are the reductant properties of dietary polyphenols, still, what may have remained disregarded is the bridge between both concepts. From this bridge a new avenue for the biological impact of polyphenols arise, circumventing the controversial issue of metabolization and the ensued potential effects on basis of very low concentrations achieved *in vivo*. The nitrate-nitrite-NO pathway may therefore provide a short cut to the signaling effects of polyphenols, linking directly diet composition and the biological outcome.

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Figure legends

Figure 1 – Short-cut for the biological effects of dietary polyphenols, in connection to the nitrate-nitrite-NO pathway, by escaping intestinal metabolization.

Figure 2 – Dietary polyphenols promote nitrite reduction to •NO in the human stomach, affording physiological relevance to an anion that was traditionally viewed as an end product of •NO metabolism. Phenolics may therefore mediate •NO-dependent biological actions through the nitrate-nitrite-NO pathway. Details on the chemical interactions of nitrite at acidic gastric pH are depicted. Under gastric conditions (pO_2, pH), nitrite is protonated into nitrous acid (HNO_2) which may undergo through different chemical pathways: 1) is reduced to •NO (*major* pathway in the presence of reductants, such as polyphenols), 2)

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yields $^{\bullet}NO_2$ (nitrating agent) that may combine with $^{\bullet}NO$ to produce 3) N_2O_3 (nitrosating agent). These chemical interactions are discussed elsewhere [68].

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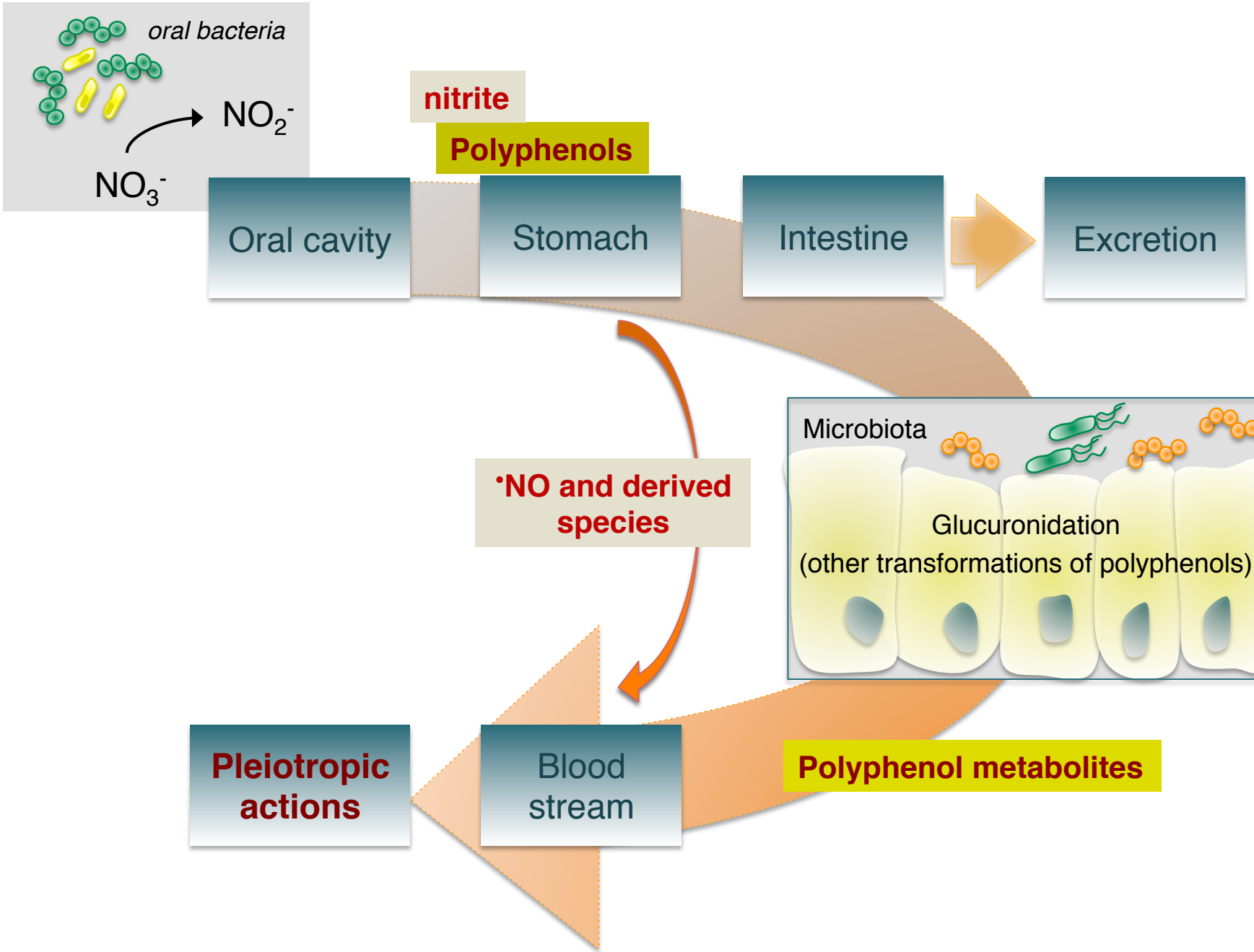

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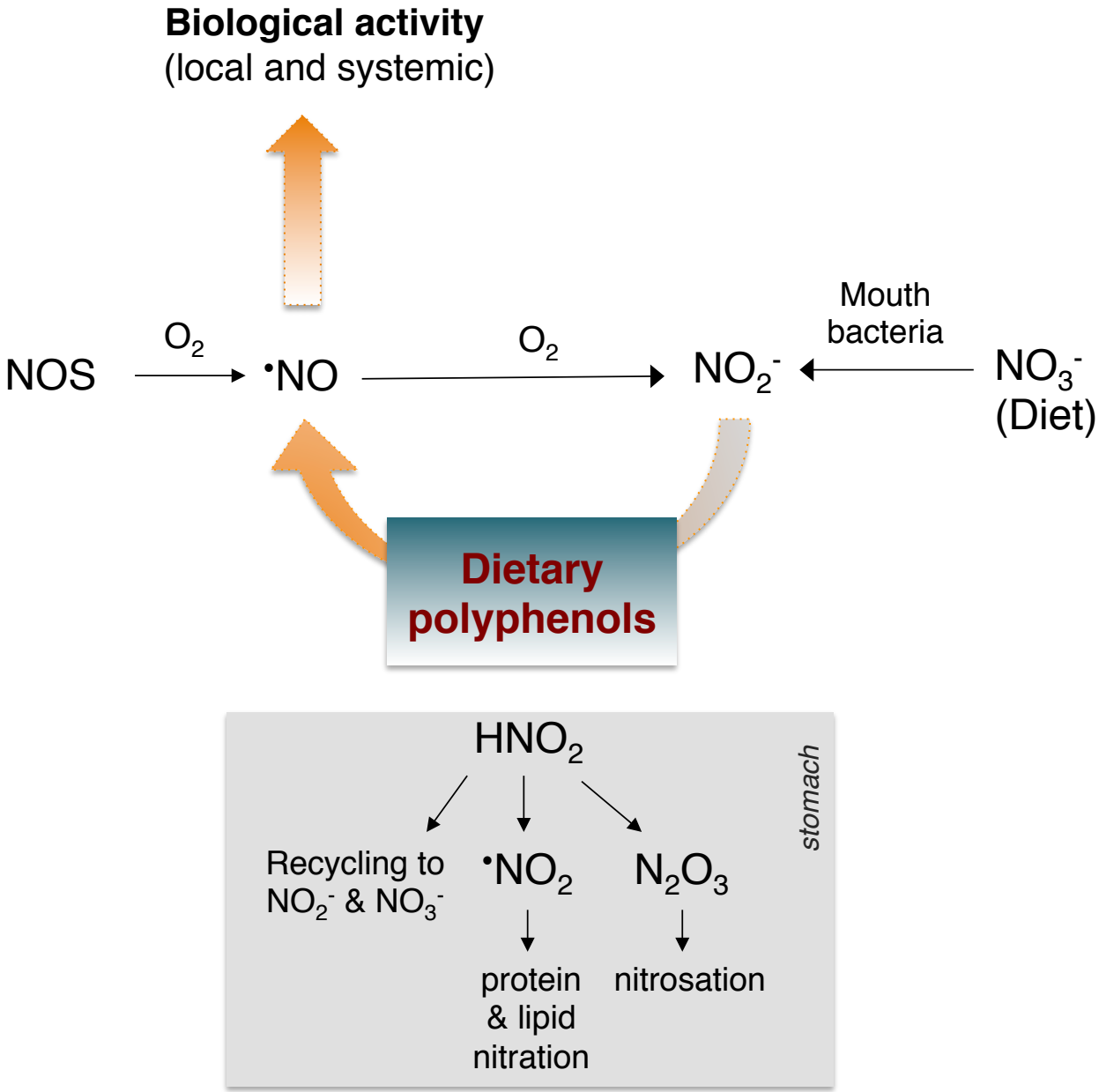

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