

Rate of deterioration and recovery of performance and discomfort (DOMS) after an athletic activity

Deterioration of performance and difficulty in recovery is an aspect in sports that weighs heavily in athletes' ability to engage in beneficial physiological adaptation to stress, marking the adaptative process their upper limit of achievement. Since in order for them to achieve high performance they must engage in frequent bouts of strenuous training, the discomfort DOMS (Delayed Onset Muscle Soreness) that irremediably follows subsequent to activity is largely dependent on the magnitude of EIMD (Exercise-induced Muscle Damage) induced inflammatory response. Performance largely depends on how the adaptive process that follows EIMD is managed. DOMS, its manifestation, must be dealt with individually according to each particular subject's sensibility to exercise, in order that enhancement of performance is favored and maladaptation is avoided.

A pattern of the adaptive process has been called Training Adaptation Syndrome and its evolution in time after a training challenge or event can be clearly appreciated from the graph below¹. New challenges should not be undertaken until the recovery has lead to reestablishment of the full ability to function. This of course is a time lapse that varies with the individual but which may in some cases exceed 48 hrs.



Also, how improvement of performance with training of the athlete and adequate recovery/adaptation is progressively

achieved, can also be appreciated in the graphs shown above and below¹, leading to the morphological reorganisation of the functional systems that physical exercise puts under stress. Only then higher performance may be mastered.



However, despite the extensive literature and investigation on the subject, the process is not well understood. This lack of clear understanding has motivated resorting to empirical methods for management of DOMS which consist mostly in applications of cold and cryotherapy, static compression, intermittent peristaltic compression, massage, nutrition, heat-related treatments, trekking poles, ultrasound and electrical current modalities, hyperoxia or hypoxia, laser therapy, spa therapy, mechanomyographical feedback, vibration therapy, acupuncture, and homeopathy. Almost all are costly and time consuming and among them only massage has shown statistically significant improvement in reducing soreness but shows only limited results in transfer to performance enhancement.

In this essay DOMS will be examined and depicted according to the best scientific research that could be found, hoping to give a clearer rendering of what it consists of and seeking a better understanding by athletes, opening perhaps better ways for performance enhancement without the risk of overreaching and falling into maladaptive outcomes. How DOMS affects the adaptation process will be then especially considered.

What is DOMS?

This question cannot be definitely answered, but recent discoveries have given a better idea of what it is in essence. To begin with it remarkably resembles a response to sepsis.



Response of muscle to exercise resembles closely to the innate immunity release of alarmins, pro-inflammatory cytokines (e.g., IL-1 β , TNF α) and of pro-inflammatory M1 macrophages, in response to sepsis; as shown in the graph above². Antagonist cytokines at the beginning of resolution (blue) phase will subsequently follow and also the release of soluble cytokine antagonists (e.g., IL-1Ra). Tissue repair at the final phase (green) involves the release of immunomodulatory type 2 cytokines (e.g., IL-10 and TGF β) and stimulation of tissue resident regulatory T cells (Treg) and M2 macrophages. Potential roles of cytokines (IL-25 and IL-33, IL-6, IL-22), can promote activation of mesenchymal fibro-adipogenic precursor (FAP) cells and immune lymphoid cells (ILCs) to promote tissue repair.

Innate Lymphoid Cells

Group	Cells	Cell markers	Characteristics
ILC1	NKC	Lin- aCD56lo.	Recognize infected cells by the absence of MHC I molecules
ILC2		Lin- CD117+/- CD127+ CD25+ ST2+, CRTH2+	Roles in allergic asthma and muscle repair
ILC3	LTi cells	Lin- CD56- CD117- CD127- NKp44+ NKp46+	Promote formation of secondary lymphoid tissues, role in mucosal immunity
	ILC22	Lin- CD56+ NKp44+ NKp46+ CD117+, CD127+	Secrete IL-17A and IL-22 cytokines

Group	Cells	Cell markers	Characteristics
Lineage negative (Lin-) cells are selected according to the methods of Ng, Fairchild [91] for the absence of specific cell surface markers.			

Table n°1

More specifically in EIMD macrophages and FAPs contribute to effective debris disposal and together with Treg cells they sustain satellite cell proliferation/differentiation and contribute to the resolution of inflammation. However, in some cases the original damaging noxa cannot be removed and maladaptive muscle remodeling takes place with persistent inflammation and recruitment of autoreactive T cells (CD4+/CD8+) ending in substitution of myofibers with noncontractile elements such as fat and fibrotic tissue³.

A significant correlation between CRP and a calculated score based on IL-1 β and IL-6 levels has been reported during EIMD⁴. The response to the two cytokines is cooperative or synergistic and the correlation was calculated using normalized values of all patients from all points in time and an appropriate model for the synergistic action of IL-6 and IL-1 β as suggested by Ganter et al⁵. Although EIMD is not generally regarded as an Acute Phase Reaction, the correlated elevation of CRP suggests that it is borderline showing evidence of systemic innate immunity response. EIMD and its

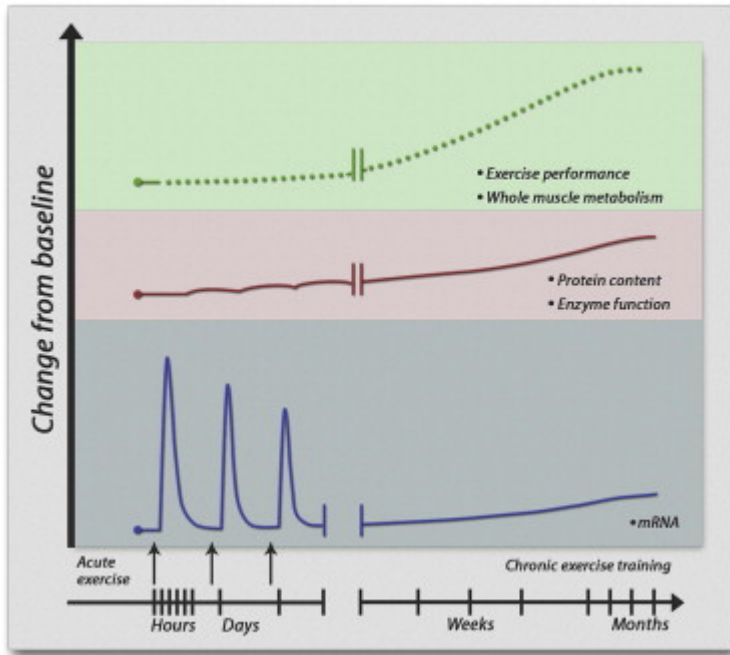
manifestation DOMS can be considered as typical aseptic inflammation.

Reductions in skeletal muscle function after intermittent-sprint exercise are often proposed to be caused by a range of peripherally-induced factors, including: intra-muscular glycogen depletion; increased muscle and blood metabolites concentrations; altered Ca^{++} or $\text{Na}^{+}\text{-K}^{+}$ pump function; increased skeletal muscle damage; pH decrement; excessive increases in endogenous muscle and core temperatures; and the reduction in circulatory function via reduced blood volume and hypohydration^{6,7}.

The adaptive response

Though a complex process, as shown above repeated episodic bouts of muscle contraction, associated with frequent exercise training, are potent stimuli for physiological adaptation. Skeletal muscle demonstrates remarkable malleability in functional adaptation and remodeling response to contractile activity over time^{8,9}. Training-induced adaptations are reflected by changes in contractile protein and function^{10,11}, mitochondrial function¹², metabolic regulation¹³, intracellular signaling¹⁴, and transcriptional responses¹⁵. The molecular mechanisms that govern the adaptation to training, involve a gross gradual alteration in protein content and of enzyme activities. These progressive changes reflect activation and/or repression of specific signaling pathways that regulate transcription and translation, and exercise-responsive gene expression. Transient postexercise changes in gene transcription, early involve genes, myogenic regulators, genes of carbohydrate (CHO) metabolism, lipid mobilization, transport and oxidation, mitochondrial metabolism and oxidative phosphorylation, and transcriptional regulators of gene expression and mitochondrial biogenesis^{15,16,17,18}. A

single bout of exercise alters the DNA binding activity of a variety of transcription factors, including MEF2^{19, 20}, and NRFs^{21, 22}. Protein stability and subcellular localization of transcriptional factor complexes within the nucleus and mitochondrion are also affected^{23, 24, 25}. Moreover, Egan et al. (2013) have recently shown that transient DNA hypomethylation of gene-specific promoter regions precedes increases in mRNA expression in response to acute exercise²⁶. These pulses of elevated mRNA during recovery in turn facilitate the synthesis of respective proteins to elicit gradual structural remodeling and long-term functional adjustments²⁷, which contribute towards maximizing substrate delivery, mitochondrial respiratory capacity, and contractile function during exercise. The net effect is optimal performance during future exercise challenges, with readaptation of homeostasis, or hormesis, characterized by biphasic dose responses of generally similar quantitative features with respect to amplitude and range of the stimulatory response that are either directly induced or the result of compensatory biological processes following an initial disruption in homeostasis²⁸. Adaptation implies altered but optimal metabolic resource allocation and eventually the establishment of an organismal state of enhanced resistance to peripheral fatigue^{29, 30}, due to diminished sustained currents (signals) able to activate metaboreceptors sensory nerve endings outside of small arterioles and venules of fascia surrounding muscles bundles^{31, 32} and readily accessible to muscle produced fatigue-mediating metabolites³³.



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The Molecular Basis of Adaptation to Exercise

Schematic representation of changes in mRNA expression (bottom panel) and protein content (middle panel) over time as a consequence of acute exercise and chronic (repetitive) exercise training. Although each individual bout of exercise is necessary as a stimulus for adaptation, it alone is insufficient to alter the muscle training-induced phenotype. Adaptation is therefore the consequence of repetitive stimuli deriving from the sum of individual exercise bouts. An individual exercise bout elicits during recovery a rapid and transient increase in relative mRNA expression of a given gene. Several fold alterations in mRNA expression from basal levels are typical and peak at 3–12 hrs after cessation of exercise, generally returning to basal levels within 24 hr. This pattern is specific to a given gene and to the exercise challenge. Translational processing together with elevation of postexercise mRNA result in a modest, parallel boost in contractile protein content. Accumulation of repeated exercise bouts elicits the gradual increase of protein as response to the pulsed increases in mRNA expression. Thus, long-term adaptation to training is due to cumulative effects of many acute exercise bouts, leading to a new functional status. Training-induced changes in protein content or enzyme function resulting in improved exercise performance (upper panel), will impose further substrate and metabolic demands. Usually protein half-lives are much longer than those of mRNA, making changes in protein content more readily noticeable than changes in transcript expression and respectively so more in training as opposed to individual bouts of exercise.

The metabolic surge of myeloid cells during the innate immunity response to intense exercise must be added to the demands of muscle contraction and compensated for, in order

for such optimal resource allocation to be attained. Immunometabolic reprogramming allows myeloid cells to meet their functional demands under the conditions existing in the surrounding microenvironment, however the organism will account for this demand and sense it as a component of healthy acute fatigue. For the initial proinflammatory conditions of neutrophil recruitment, phagocytosis and NET formation) observed during intense exercise and afterwards during recovery (monocyte recruitment), glycolysis provides a rapid means for the generation of ATP and biosynthetic intermediates. Energy required for these and other functions are provided from a high rate of glycolysis³⁵. With relatively few mitochondria, neutrophils can receive electrons from glycolysis via the glycerol-3-phosphate (G6P) shuttle into complex III in order to maintain $\Delta\psi_m$ but hardly coupled to ADP phosphorylation by action of Complex V (F_1/F_0 -ATPase). Although less efficiently, ATP is yielded faster by glycolysis to meet the demand; a precipitous ATP production while catabolic efficiency is sacrificed. But G6P can also enter the pentose phosphate pathway (PPP), a spinoff of glycolysis that generates reducing equivalents of NADPH necessary for the microbicidal mechanisms regulated by NOX and riboses essential for nucleotide synthesis, playing also a significant role in the cellular redox status³⁶. This ensures that neutrophils can function in an inflammatory environment where the oxygen tension may be low or even absent^{37,38}. Though the glycolytic process can also proceed aerobically³⁶.

Favorable recovery from exercise should be characterized by a prompt diminishing sense of fatigue and should not consist in more than –metabolite mediated– metabolic signaling, to prevent further dangerous energy consumption (muscle damage, rhabdomyolysis, pathological myocardium remodeling and cardiac arrhythmia³⁹, chronic fatigue, adrenal insufficiency⁴⁰) beyond diminishing returns and non-adaptive energy spending⁴¹. As

noted, increased resistance awareness to peripheral fatigue should be perceived with progressive training.



cfDNA

Findings of presence of cell free DNA in plasma during recovery from acute exercise have shed more light on the unanswered aspects of the section above: What is DOMS? The source of cfDNA remains speculative, however, a rapid cfDNA accumulation due to active mechanisms seems likely rather than passive cell death events as previously suggested, however eventually ending in special forms of cell death.

Neutrophils can also kill pathogens extracellularly by releasing neutrophil extracellular traps (NETs; Brinkmann et al., 2004)⁴³. An evolutionarily highly conserved first line defense glycolytic⁴⁴ mechanism of cellular transformation in which the nuclear membrane breaks down, DNA mixes with cytoplasm and the whole assemblage is jettisoned from the cell providing a key ingredient for pus and other exudates. They represent a physical structure to ensnare and immobilize bacteria and other micro-organisms in a mesh or net-like structure. Like a fly in a spider's web, the organisms are stuck and can be further attacked by the antibacterial proteins that stud the DNA^{45, 46, 47}. Interestingly, histones have anti-bacterial activity, providing another example of a nuclear molecule doing double duty for the cell, fulfilling important functions in both the intracellular and extracellular space⁴⁸. The importance of NETs in microbial defense is underscored by their presence in pus. Superficial infections produce pus. For centuries, as Pisetsky (2011) pointed out in a recent review, pus with a high viscosity was regarded as "good" because it resolved the infection. Now we

know that pus consists mostly of neutrophils surrounded by NETs.

Molecularly, the few events that have been shown to be required, sequentially, are the production of ROS, the migration of the protease neutrophil elastase (NE) and later myeloperoxidase (MPO) from granules to the nucleus, the processing of histones, and eventually the rupture of the cell. Eventually, NETs are removed during the resolution of inflammation. The impact of NETs derives from the combined antimicrobial activities of granular components, histones, and some cytoplasmic proteins. Eosinophils and mast cells, which are granulocytes closely related to neutrophils, granulocyte homologues in lower vertebrates, and even plants release extracellular traps. NETs are the results of a unique form of cell death that morphologically is characterized by the loss of intracellular membranes before the integrity of the plasma membrane is compromised. To release NETs, activated neutrophils undergo dramatic morphological changes. Minutes after activation, they flatten and firmly attach to the substratum. After 1 hr, the nuclear envelope disaggregates into vesicles and the nucleoplasm and cytoplasm form a homogenous mass. Finally, the cells round up and seem to contract until the cell membrane ruptures and the interior of the cell is ejected into the extracellular space, forming NETs (Video). Notably, despite the intermixing of cellular compartments, during the last phase of NETosis, <30 proteins are present in NETs. Most of them originate from granules, few are from the nucleus, and cytoplasmic NET components are rare.

Time-lapse recording of neutrophils undergoing NETosis. Neutrophils were stimulated with PMA, and a z stack was generated for 2 hrs and 25 min on a confocal microscope. Directly labeled antibody fragments against NE (green) and chromatin (red) in the supernatant depict formation of NETs in the final phase.



cfDNA release

In addition to their function in the defense against infection, NETs may contribute to the pathogenesis of rheumatic disease. NETs are present at sites of tissue injury in vasculitis, promote thrombosis (an important concomitant of many connective tissue diseases) and represent an important source of DNA to form immune complexes and drive type I interferon production^{49, 50, 51, 52, 53, 54}. Coagulation is a way to reduce blood loss after injury, but it also represents a primitive innate immune response that limits microbial spreading⁵⁵. Coagulation is an example of how the amount of NET formation can determine a “good” or “bad” outcome. NETs participate in timely clot formation, but if present in excess they induce massive coagulation that can stop the blood supply of organs, causing severe ischemia.

Acute exercise at different intensities elicits varied effects on oxidative stress, shear rate, and endothelin-1 (ET-1) that do not appear to mediate changes in endothelial function measured by artery flow-mediated dilation (FMD)⁵⁶. However, an elevated cfDNA level in both non-athletes and athletes can be associated with markers of vascular endothelial dysfunction such as cytokines IL-1 β , IL-6 and TNF- α , hsCRP, oxidized lipoproteins as well as reactive oxygen and nitrogen species, etc. The main source of cfDNA is apoptosis of endothelial cells and circulating endothelial progenitor cells as well as NETosis of immune cells which are programmed to move toward sites of vascular injury⁵⁷.

The fact that road cycling events in certain ambient conditions (hot conditions) have resulted in increased concentrations of platelet, platelet activation, coagulation, and fibrinolytic markers in both men and women it is of concern, despite that the fibrinolytic system markers also increased, which appears to balance blood hemostasis and may

prevent clot formation during exercise^{58, 59}. In view of the recent findings of persistent NET formation during exercise and that hypercoagulability persists for hours and up to a day after extreme exertion⁶⁰, this aspect merits further investigation and caution should be adopted when exercise must be performed under extreme conditions.

Exercise-induced inflammation and sports performance

EIMD and its associated inflammatory response is of paramount importance for athletes' performance for two basic reasons:

- the rate of deterioration and recovery of performance and discomfort (DOMS) after an athletic activity is largely dependent on the magnitude of EIMD-induced inflammatory response.
- it affects the frequency of training stimuli, that is, the time needed for optimal recovery in between practices, official events, and/or an event and a practice.

A >20% decline of the force-generating capacity of muscles exhibits a close association with the magnitude of muscle damage and its associated inflammatory response^{61, 62, 63}. Smaller reduction of muscle strength is usually not accompanied by histological evidence of EIMD⁶⁴. Paulsen et al suggested that strenuous isolated eccentric activity produces a greater degree of EIMD than the so-called eccentrically biased activities such as downhill running or intense-level running. Evidence indicates that the magnitude of force, the degree of lengthening, velocity of movement, and overall volume of eccentric load are probably the most important factors dictating the rate of muscle injury in response to exercise^{65, 66}. In fact, it has been shown that EIMD induced by a football match is associated with the number of explosive

types of movement that incorporate a strong eccentric component^{67, 7}.

In cases of mild performance decline, defined by Paulsen et al.⁶⁴ as a drop of strength by <20%, creatine kinase activity (CK) in blood remains <1,000 U/L, inflammation is limited, and recovery is usually fast (within 12–48 hrs) independent of type of exercise^{68, 69, 70, 71, 72}. In the case of moderate performance decline, defined by Paulsen et al.⁶⁴ as a drop of strength by <20%–50%, myofiber necrosis may be observed (mostly in high-respondents)⁶⁸, CK exceeds 1,000 U/L, the inflammatory response is more intense with leukocyte infiltration into the injured muscle, and degradation of structural and contractile proteins may be evident and recovery is usually completed within a week^{73, 74, 68, 75, 76}. In the case of severe performance decline, defined by Paulsen et al.⁸ as a drop of strength by >50%, necrosis is usually observed in parts of the myofibers, CK may exceed 10,000 U/L, soreness is quite high, muscle swelling is evident, the inflammatory response is intense and characterized by a marked accumulation of immune cells into the traumatized tissue, and recovery usually takes 1–3 weeks or even longer if strength loss is >70%^{77, 78, 68, 79, 80, 81, 82, 83, 84}. In severe EIMD, increased proteolysis and muscle disruption is observed, even during early recovery, due to disturbances in calcium homeostasis⁸⁵. In these three scenarios, athletes may need to use different recovery strategies in order to be able to train or compete as soon as possible. Recovery treatments may be classified in four major categories:

1. pharmacological (e.g., inflammatory agents, NF-κB inhibitors, estrogen therapy, phosphodiesterase inhibitors, ACE inhibitors),
2. nutritional approaches and supplements (e.g., antioxidants, herbal remedies, alcohol ingestion, ω-3-

fatty acids, β -hydroxy- β -methylbutyrate, protease supplementation, nucleotide supplementation, tea consumption, beetroot, caffeine, creatine, enzyme supplementation, and carbohydrate and/or protein supplementation),

3. rehabilitation and physical therapy methods (e.g., cryotherapy, heat-related treatments, compressive loading techniques, trekking poles, ultrasound and electrical current modalities, massage, hyperoxia or hypoxia, laser therapy, spa therapy, mechanomyographical feedback, vibration therapy, acupuncture, and homeopathy), and
4. exercise-related treatments (e.g., stretching and low-intensity exercise), and numerous studies and reviews have examined their effectiveness in reducing EIMD and accelerating recovery^{86, 87, 88, 89, 90}.

Of those, massage is promising in reducing soreness but shows limited results in shortening the time needed for muscle recovery or inducing performance enhancement and lack of an observed effect on lactate clearance^{91, 92, 93, 94, 95}. Hemmings et al. raised questions about the efficacy of massage in a study of physiological and psychological recovery stating that massage may be beneficial psychologically but not physiologically⁹⁶. Nelson⁹¹ also noted that observations of massage effects were more easily shown in the psychological than the physiological domain. McGlone et al. described the neurons as, a class of low-threshold mechanosensitive C fibers that innervate the hairy skin represent the neurobiological substrate for the affective and rewarding properties of touch⁹⁷.

These recovery approaches aim to reduce swelling, improve blood flow, pain sensation, immune cell recruitment, and/or improve healing by activating satellite cells and anabolic factors as well as improving tendon healing. However there seems to be little scientific support towards their efficacy

regarding those immediate aspects and quite uncertain is whether the more important aspects in effective and timely performance recovery/adaptation can in essence be managed. So, it results that today no general guidelines exist regarding the treatment of EIMD and performance recovery.

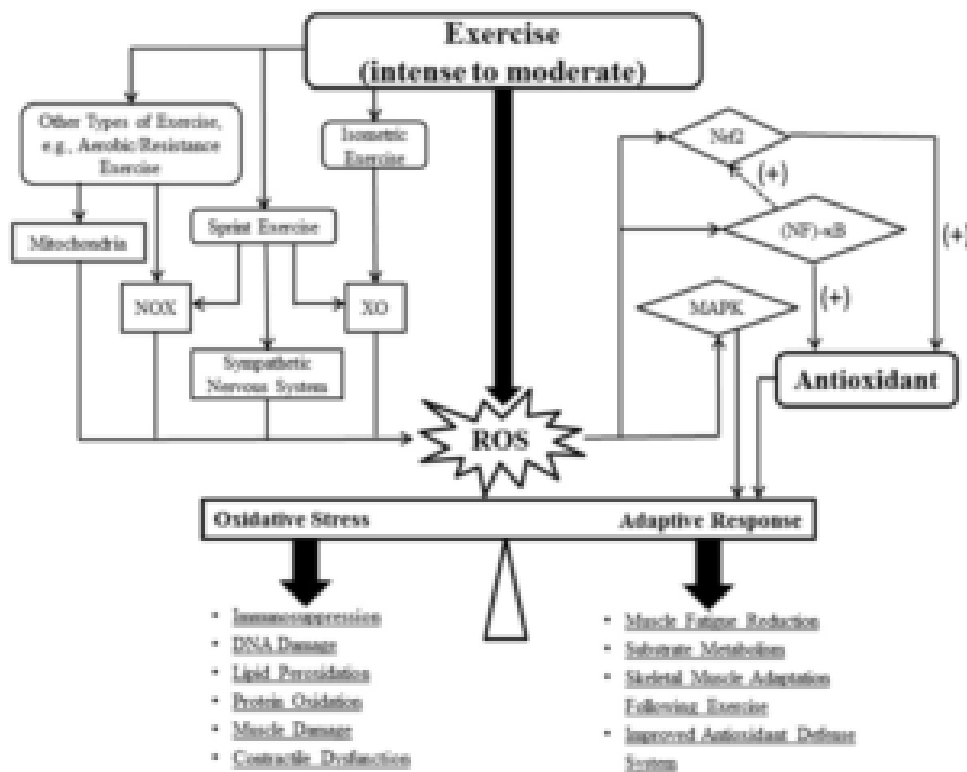
Certain concerns have been raised regarding the use of anti-inflammatory agents based on reports suggesting that these products not only disrupt the inflammatory response but also hinder the adaptive response to training. However, the periods during which anti-inflammatory agents are used need to be discerned. There are training periods during which athletes need to compete and train with very high frequency (e.g., in-season training), and cessation would be beneficial. In contrast, there are periods during which athletes train aiming for long-term adaptations, and anti-inflammatory agents may disrupt both EIMD and training adaptations. In the latter case, athletes should rethink about using such recovery approaches. This seems to have been substantiated in 24 hour ultramarathon races, where no beneficial effects were measured from anti-inflammatory agents in reducing EIMD, or DOMS. To the contrary, ibuprofen use was linked to mild endotoxemia, kidney dysfunction, and systemic inflammation⁹⁸.

Now it is also well-understood that athletes may be low, moderate, and high-respondents to EIMD, a fact that also explains the substantial interindividual variability in the responses seen following various types of exercise protocols used to induce muscle microinjury^{99, 79, 100, 86, 101, 102}. This phenomenon may be attributed to a number of factors such as age,^{103, 104, 105} preconditioning,^{106, 107} gender,^{108, 83} genetics,^{109, 110} physical conditioning level,^{67, 111, 112} and joint range of motion¹¹³. Therefore, sport practitioners should determine whether their athletes are low, moderate, or high respondents in order to establish individual normative values for each one, and should avoid absolute comparisons among athletes or

generalizations.

It must also be mentioned that preconditioning, a phenomenon also known as “repeated bout effect,” may protect athletes from EIMD, that is, after a first session of damaging exercise, skeletal muscle tissue adapts and is less vulnerable to injury in subsequent sessions of the same type of exercise but in a different group of muscles^{114, 115}, exhibiting less EIMD, inflammation, oxidative stress, leukocyte infiltration, and strength loss^{76, 116, 114, 50, 117}.

Adaptive antioxidation during exercise



He F. et al

ROS produced by leukocytes (due to activation of NADPH oxidase and enzymes such as xanthine oxidase and cyclooxygenase-2) in response to the action of cytokines released by injured muscle as well as by invading neutrophils not only offer antiseptic defense to the muscle but also result in drastic disturbance of muscle's redox status^{118, 119, 120}. ROS release during

postexercise recovery may cause a secondary damage to both afflicted and healthy adjacent myofibers (due to oxidation of muscle's protein and lipid molecules) despite a rise in muscle's antioxidant reserves¹²¹. Reduced glutathione (GSH) is one of the main antioxidants of muscle and is used to neutralize ROS thereby producing its oxidized form (GSSG), a process that results in marked perturbation of redox status in myofibers¹²². It has been suggested that the GSH/GSSG couple functions as a key-controller of important redox-sensitive intracellular signaling pathways that lead to cytokine synthesis and release by the injured muscle to activate immune cell recruitment and adhesion such as nuclear transcription factor κ B (NF κ B) and mitogen activated protein kinases (MAPK)¹²³. Recent evidence from Michailidis Y. et al. indicated that by attenuating the decline of GSH/GSSG following extensive muscle damage induced by eccentric exercise through administration of a potent thiol-based antioxidant, that is, N-acetylcysteine (NAC), macrophage infiltration in injured muscle, was reduced by ~30% and this response was accompanied by a blunted activation of proinflammatory cytokines and NF- κ B and MAPK signaling. Supplementation of antioxidant vitamins did not affect CD4⁺, CD8⁺, naive T cells, NK cells, and proinflammatory cytokines responses following intense eccentric exercise¹²⁴. In contrast, in vivo and in vitro studies have shown that alterations of GSH/GSSG status via administration of NAC may result in reduced neutrophil chemotaxis¹²⁵ and macrophage accumulation¹²⁶, by attenuating the NF- κ B-dependent proinflammatory cytokine expression and release^{127, 128, 129}, as well as increased lymphocyte numbers and activation by inhibiting their apoptotic rate¹³⁰ suggesting that immune responses in inflammatory states may be redox-dependent. However, this possibility has not been explored in humans in inflammation induced by muscle-damaging exercise. Elucidation of this possibility will shed light to the

mechanisms involved in skeletal muscle trauma and aid in the development of potential treatments for diseases characterized by muscle inflammation. In this study Michailidis Y. et al. utilized NAC supplementation to enhance GSH stores during recovery from a very intense eccentric exercise protocol performed on an isokinetic dynamometer hypothesizing that NAC administration alters immune cell responses following exercise-induced muscle damage.

Surprisingly, oxidative stress during 24 hour ultramarathon racing was found to be modest and not related to elevations in plasma cytokines^{98, 131}. Post-race blood leukocyte mRNA expression for IL-10 was unusually high in comparison to very low values for IL-8 reflecting the immune system's effort to dampen inflammation.

Exercise Performance


Dietary nitrate has the potential to reduce blood pressure, lower the oxygen cost of exercise, and, at least in some circumstances, enhance exercise capacity. Amongst the nutritional treatments for enhancing exercise performance, it has been studied abundantly and several points are worth to be pointed out in favor of adopting its use.

With regard to the effect of nitrate on indices of exercise performance in healthy volunteers, the literature appears consistent in showing that 2–6 days (or up to 15 days) of supplementation can increase indices of performance during high-intensity constant work-rate exercise and maximal incremental exercise^{132, 133, 134}. The effects of acute supplementation on performance are less consistent, with some studies showing a positive effect^{135, 136, 137, 138}.and others showing no effect^{139, 140}. It is likely that the efficacy of acute nitrate supplementation will depend on several factors such as the age, health, diet, and fitness/training status (including

muscle fiber type proportions, capillarization, and baseline plasma [nitrite]) of the subjects tested; the intensity, duration, and nature of the exercise task; and whether the exercise is performed in normoxia or hypoxia. Acute nitrate intake may rapidly influence vascular tone and peripheral tissue oxygenation^{134, 135}, but more time may be necessary to permit changes in mitochondrial and contractile proteins to influence exercise performance^{141, 142}. Whether longer-term nitrate supplementation may support or augment (or even hinder) the physiological adaptations to training is presently unknown.

The duration of continuous maximal exercise for which nitrate appears to be ergogenic is in the range of 5–30 min^{132, 133, 136, 143}. There is limited evidence that nitrate is beneficial for longer duration exercise (>40 min) performance, at least when administered acutely^{139, 144}. This may be related to the lower intensity of such exercise and the associated reduced likelihood of the development of local mismatching of perfusion to metabolic rate in muscle (i.e. loci that are relatively hypoxic and acidic). Whether nitrate supplementation may be ergogenic during very high-intensity continuous or intermittent exercise has not been systematically evaluated. However, two studies indicate that high-intensity intermittent exercise performance might be enhanced by nitrate supplementation^{143, 138}.

Mechanisms in skeletal muscle performance

The results of¹³³ were important in demonstrating that the reduction in the whole-body oxygen cost of exercise following nitrate supplementation is consequent to changes in muscle energy metabolism. The proportional sparing of  and PCr

reported by¹³³ indicated that nitrate supplementation may alter the energy (adenosine triphosphate [ATP]) cost of muscle power production. The authors suggested that this could occur by the possible effects of NO on the sarcoplasmic reticulum calcium (Ca^{2+}) ATPase or the actin-myosin ATPase^{145, 146}. A lower ATP cost of force production would blunt the changes in intramuscular substrates and metabolites that stimulate mitochondrial respiration (e.g. PCr, ADP, P_i)^{147, 148}. and could explain the lower oxygen cost of exercise. Interestingly, the depletion of muscle PCr and the accumulation of P_i and ADP have been linked with the process of muscle fatigue during high-intensity exercise¹⁴⁹. The blunted changes in energy substrates and metabolites might therefore help to explain the improved exercise tolerance observed following nitrate supplementation. An alternative explanation for the coincident reductions in steady-state \dot{V}_{O_2} and PCr is that nitrate supplementation simultaneously improves muscle oxygenation (thus sparing muscle PCr)¹⁵⁰ and improves mitochondrial efficiency (thus lowering \dot{V}_{O_2}).

Evidence for positive effects of nitrate supplementation on both mitochondrial efficiency¹⁴¹ and muscle contractile function¹⁴² has recently been presented. Filip J Larsen et al. asked 14 healthy volunteers to consume 0.1 mmol/kg BM/day of sodium nitrate or a placebo for 3 days, after which a muscle biopsy was taken and a submaximal exercise test was completed. Following nitrate supplementation, the expression of adenine nucleotide translocase (ANT), a protein involved in mitochondrial proton conductance, was reduced, thus reducing leak respiration and improving the efficiency of oxidative phosphorylation. Nitrate supplementation resulted in a 19 % increase in the mitochondrial P/O ratio (the amount of oxygen consumed per ATP produced), which was closely correlated ($r = -0.80$) with the reduction in whole-body \dot{V}_{O_2} during

submaximal cycling. These results indicate that the reduced x during exercise following nitrate supplementation is related to a reduced leakage/slippage of protons across the inner mitochondrial membrane. The authors speculated that nitrate supplementation might result in an increased inhibition of cytochrome c oxidase by NO^{141,151, 152} which might be sensed by the cell as mild hypoxia, initiating signaling mechanisms that result in a downregulation of ANT and improved mitochondrial efficiency. Interestingly, in contrast to 3 days of in vivo nitrate administration, the acute application of nitrite to isolated mitochondria in vitro had no acute effect on the P/O ratio. This finding suggests that several days of nitrate treatment may be required for the induction of changes in the expression of relevant mitochondrial proteins such as ANT.

However, the effects of nitrate supplementation on muscle function may not be confined to the mitochondria. Hernández et al. have reported improvements in muscle Ca²⁺ handling and contractile function in mice fed sodium nitrate in water for 7 days compared with age-matched controls that received water without added nitrate. In particular, in fast-twitch muscle fibers, nitrate supplementation increased myoplasmic free Ca²⁺ concentration at stimulation frequencies from 20 to 150 Hz, effects that were related to the increased expression of calsequestrin 1 and the dihydropyridine receptor, proteins that are involved in Ca²⁺ handling. These striking effects on intracellular Ca²⁺ handling resulted in significantly increased contractile force at 50 Hz or less and a faster rate of force development at 100 Hz stimulation. There were no effects of nitrate supplementation on muscle proteins or contractile force in slow-twitch muscles. The authors concluded that dietary nitrate intake in humans may increase muscle function during normal movement. The results of Hernández et al. are consistent with the suggestion¹³³ that the effects of nitrate on

muscle efficiency may be explained, at least in part, by extramitochondrial mechanisms.

In addition to evoking these intracellular effects, there is recent evidence that nitrate supplementation might also enhance blood flow to contracting muscle. Ferguson et al¹⁵³ fed beetroot juice (or a water placebo) to rats for 5 days, and then measured blood pressure and hind limb muscle blood flow during submaximal treadmill running. Exercising blood pressure and blood [lactate] were significantly lower following nitrate feeding, and there was a striking (38 %) increase in muscle blood flow between nitrate-fed and placebo-fed rats. The greater muscle blood flow was directed preferentially towards hind limb muscles expressing a high fraction of type II muscle fibers. Muscle oxygen delivery was therefore substantially elevated in the low oxidative, highly fatigable fibers, such that oxygen might be considered to be more appropriately distributed across and within the active muscles. This might be expected to reduce substrate-level phosphorylation, improve metabolic control and exercise efficiency, and be advantageous to performance¹³². In a subsequent study, the same authors reported that microvascular oxygen pressure fell less rapidly following the onset of electrically evoked contractions of the spinotrapezius muscle of rats fed beetroot juice compared with those fed water¹⁵³. This is consistent with a greater oxygen driving pressure across the transition from rest to exercise. Those studies indicate that nitrate supplementation, which may reduce both the ATP and oxygen cost of muscle contraction simultaneously increases muscle oxygen delivery. The net result is a higher ratio of oxygen delivery to oxygen utilization, which would be expected to reduce the muscle metabolic perturbation and be conducive to muscle fatigue resistance.

In conclusion efficacy of nitrate might well depend on factors such as the type of subject, including age, diet, and health

and fitness status; the intensity, duration, and nature of the exercise challenge; and the dose applied and duration of the nitrate supplementation regimen.

Microenvironment approach for managing recovery/adaptation

The abundant research on the subject points to an organismic response to the challenges that exercise impose at the local musculoskeletal level. The approach of improving pH, redox potential and electric fields of the contractile myofibrillar units is a novel way of dealing with DOMS and in abbreviating recovery period of athletes and enhancing adaption in the longer term.



Bioactil Ultra applied topically has been shown to relief DOMS in a very short period and in diminishing peripheral fatigue, while at the same time inducing a pleasant central sensation of well-being. Therefore, it is useful in managing both peripheral and central fatigue and making the return to work and daily life of athletes a common routine. Non-professional athletes then can enjoy practicing sports without interference with daily routine.

In elite athletes Bioactil Ultra can accelerate and facilitate peak performance status with shorter intervals of rest between training bouts and solving any hindrance form trigger-points that might need lengthy recovery by conventional means.

In overt lesions Bioactil Ultra has shown to be quite a powerful tool to resolve swelling, pain and inflammation while stimulating resolution of healing.

While activation of coagulation and fibrinolysis is a constant finding in all runners post-marathon, wearing compression socks was shown to reduce fibrinolytic activity, as demonstrated by lower D-Dimer concentrations. Compression may reduce exercise-associated haemostatic activation when

completing prolonged exercise¹⁵⁴.

 Bioactil Gel Refrescante	 Optimal combination for recovery
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As shown in the image above, combining the use of Bioactil (already quite satisfactory on its own) with compression socks is a good evidence-based practice that enhances rapid and safe recovery. Athletes following such method have manifested high satisfaction in speed and quality of peripheral recovery, plus a boosting and pleasant soothing central sensation during the process.

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Remineralization and healthy gums for a strong heart



Plusalus is the social network of a scientific group (BioAcyl Corp) dedicated to apply proprietary medical knowledge accumulated for over a decade and which has formulated an advanced system of oral care reuniting proprietary experience with knowledge published in the scientific literature during the last 35 years.

An innovative delivery system into gums and teeth, the mouth and pharynx of the agents identified in the research that can balance bacterial populations and dissolve biofilm is fundamental towards the efficacy of the system. The vehicle which is fully edible serve towards that effect. The finished product can be used to treat problems or for daily care that can span for a lifetime. .

The new method has finally turned away from the failed and over-hyped intent of killing bacteria enduring for over a century. So through the extent of the present text you will not meet at any moment such foolish claim.

On one aspect this new approach is oriented towards modifying the morbid traits of *Streptococcus mutans sobrinus* seeking the scope of modifying the ability that this bacteria has to form

dental plaque and prevent its adherence to it. From plaque this bacteria produces the acid that will dissolve enamel (acidogenic bacteria)..

The other aspect tends to restore the correct microenvironment that promotes healthy normal physiological conditions in the mouth.

The latter will not only diminish cavities drastically, but also it makes possible to normalize the sub-gingival plaque (present under the gums) and prevent the formation of biofilm there where multiple strains of anaerobic and microaerophilic bacteria cooperate to attack the gums undermining their tissue; especially the ligament that holds the tooth to the bone causing gum disease. Biofilms are shields (like a camping tent) that colonies of bacteria secrete in order to isolate themselves from antiseptics and antibiotics. Such bacteria become insensible to any attacks thrown onto them with the purpose of annihilating them.

Scientists at BioAcyl Corp stress that it has been disregarded for almost 4 decades the fact that attack to healthy tissue is only possible if the bacteria in plaque are able to modify the microenvironment to one that will favor them. Contrariwise such bacteria could not survive in a physiological microenvironment and are unable to populate the subgingival area. So that something has to change to the physiological state for the attack to take place. If bacteria have successfully changed the healthy microenvironment and colonized the area, direct anti-biofilm action by the agents discovered at BioAcyl Corp plus restoring the physiological conditions of the microenvironment are the most effective ways for dealing with gums and teeth problems. The physiological system devised at BioAcyl Corp is a fresh and real option in dealing with simple or as well serious problems of the oral cavity.

Other agents helping in such scope have been known for quite sometime. These are the so called alditols or sugar derived

alcohols. Contrary to popular belief the great majority of sugars do not produce caries. To be able to produce caries a sugar must be recognized by the acidogenic bacteria and internalized in the microbe and turned into acid. Sugars most commonly have 4 to 6 carbons, some may contain 9. The useful ones that are not fully identified by bacteria are Eritritol, Xylitol and sorbitol and respectively in order of 4, 5 and 6 carbons. The most similar to common sugar is sorbitol differing only in how OH radicals are disposed stereochemically; yet it is quite less cariogenic than common sugar. Eritritol and Xylitol are powerfully anticariogenic. But very interestingly, when provided in place of sugar they trick the bacteria to produce defective glycoproteins used by the community of bacteria to arm the biofilm. So that formation of the shield is incomplete weak and in the end unsuccessful. No longer can bacteria guard themselves to survive as a modified community under a membrane. Xylitol has been known now for its anti-cavity effect for 35 years has been tested for its anti-biofilm effect. Eritritol is more recently also known for such actions. It is commonplace for combinations of the alditols to be used in practice Kauko K. Mäkinen 2010 .

Among other effects alditols stabilize calcium phosphate from saliva. Xylitol in particular can form complexes with calcium displacing water molecules from the solvation sphere of action of the hydroxyl groups of the alditol and keeping the cation (Ca^{++}) soluble in the plaque and saliva. Two or more hydroxyls of the alditol can displace 2 molecules of water from the solvation sphere of the cation Mäkinen, Söderling 1984 Angyal 1972 . It is also possible to facilitate calcium absorption, independent of the vitamin D effect. in the intestine by ingesting Xylitol Hikorazu et al. 2011. Some studies have demonstrated remineralization of deep caries (dentinal caries) using Xylitol K K Mäkinen 1995. Other effects (specific and non-specific) of Xylitol tend to restore the physiological environment of saliva on its own account rich in calcium

phosphate, mucins and proteins J Chirife 1984 that help restore the salivary film SAM Vacca and Smith 2000 and remineralization of teeth. The most important is the affinity that Xylitol has for water and the humectant and protecting power on tissues. Finally its sweetness is equal to the one of sugar and it will stimulate salivation, that by itself helps to remineralize.

BioAcyl agents act on the normalization of the microenvironment keeping a physiological pH, balanced redox state and energy supply. Taken together a system has been perfected that consists of Periactil Precondition and Periactil. The first component is used with a brush and the second is applied on teeth and gums by distributing it with the tongue.. Mouthrinse is banned since it can annul the protection conferred by Periactil gel which is mucoadherent. Periactil is edible and Periactil Precondtion is totally harmless if swallowed. The remaining Periactil in the mouth can be swallowed to protect the throat.

The system is able to deliver superior results by means of normalizing microenvironments and balancing the microbiota that normally populates the oral cavity. Such results are decidedly NOT obtained by trying to kill bacteria.

Cardiovascular benefits

Although the role of oral microbiota in disease is well studied, specific contributions to host health are not well defined. The entero-salivary nitrate-nitrite-nitric oxide pathway, which can positively affect nitric oxide (NO) homeostasis, represents a potential symbiotic relationship between oral bacteria and their human hosts.

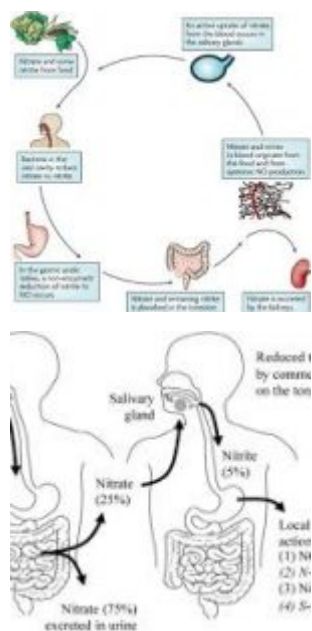


Fig. n°1

The entero-salivary nitrate-nitrite-NO pathway. Twenty-five percent of the ingested dietary nitrate is recycled to the saliva, and 20% of the nitrate in saliva is converted to nitrite by oral commensal bacteria. Approximately 5% of the originally ingested nitrate is swallowed into the stomach, and provides for NO activities in various forms. (1) NO for local vasodilation, mucus formation, and antimicrobial activity; (2) N-nitrosoamines for local carcinogenesis; (3) Nitrite for nitrite pool and transnitrosylation in the peripheral tissues; (4) S-nitrosothiols for transnitrosylation in the peripheral tissues.

The gaseous free radical NO, which is endogenously produced in vascular endothelial cells, neurons and immune cells, plays a critical role in various physiological processes, including vascular homeostasis, neurotransmission, and host defense mechanisms, respectively. It appears that we have at least two systems for affecting NO production/homeostasis. The first is

through the classical *L*-arginine-NO pathway. This is a complex and complicated five-electron oxidation of *L*-arginine and if any of the co-factors become limiting, then NO production from NOS shuts down, and in many cases, NOS produces superoxide instead Tomasz J. Guzik et al. 2002. The enzymatic production of NO during normoxic physiological conditions proceeds very efficiently. However, in disease characterized by oxidative stress essential NOS cofactors become oxidized, NOS uncoupling, or conditions of hypoxia where oxygen is limiting, this process can no longer maintain NO production Ulrich Förstermann, Thomas Münzel 2006. Therefore, it can be argued saliently that there must be an alternate route for NO production. It is highly unlikely that nature devised such a sophisticated mechanism of NO production as a sole source of a critical signalling molecule. This alternate route involves the provision of recycling nitrate and nitrite and their respective reductions down to NO. The two-electron reduction of nitrate to nitrite occurs during anaerobic respiration through symbiosis with obligate and/or facultative anaerobic commensal bacteria that reside in the crypts of the posterior third of the tongue Jon O. Lundberg et al. 2004. This process mammalian cells are unable to perform, Nitrite reduction to NO can occur in a much simpler mechanism than that of nitrate. The 1-electron reduction of nitrite can occur by ferrous heme proteins (or any redox active metal) through the following reaction: $\text{NO}_2^- + \text{Fe}^{(\text{II})} + \text{H}^+ \leftrightarrow \text{NO} + \text{Fe}^{(\text{III})} + \text{OH}^-$. Therefore the entero-salivary nitrate-nitrite-NO pathway is a NOS-independent, and oxygen-independent pathway to NO formation that is a vital alternative to produce bioactive NO, particularly during periods of hypoxia.



Fig n° 2

Continuous availability of NO is essential for cardiovascular system integrity. In the circulation, NO is an important regulator of vascular tone and blood pressure, and inhibits oxidative stress, platelet aggregation, and leukocyte adhesion. NO insufficiency is strongly correlated with cardiovascular risk factors, is causal for endothelial dysfunction, and serves as a profound predictive factor for future atherosclerotic disease progression and cardiovascular events. Dietary nitrate, obtained primarily from green leafy vegetables and beets, is rapidly absorbed from the upper gastrointestinal tract into the bloodstream, where it mixes with the nitrate formed from the oxidation of endogenous NO produced from mammalian NOS. Up to 25% of this nitrate is actively taken up by the salivary glands and concentrated up to 20-fold, reaching concentrations approaching 10 mM in the saliva. Because the plasma levels of nitrite are highly dependent on the amount of salivary nitrate and its reduction to nitrite, the use of an antibacterial mouthwash Mirco Govoni et al. 2008 and frequent spitting of saliva consequently decrease the plasma levels of nitrite. Using a single ingestion of 500 ml beetroot juice [22.5 mmol ($\sim 0.35 \text{ mmol kg}^{-1}$)], Webb et al. demonstrated a more acute and more profound fall in both systolic BP ($\sim 10.4 \text{ mmHg}$, baseline of $\sim 108 \text{ mmHg}$) and diastolic BP ($\sim 8 \text{ mmHg}$) compared with placebo (500 ml water, crossover study) Andrew J Webb 2008. Importantly, the beetroot juice did not contain any detectable nitrite, and whilst plasma nitrate levels were already increasing by 30 min, the BP did not start to fall until the plasma nitrite concentration started to rise, with maximum changes in both occurring at $\sim 2.5\text{--}3 \text{ h}$, reflecting the time to produce nitrite from nitrate and for it to accumulate via the enterosalivary circulation (described above). Indeed interruption of this process by asking volunteers to spit out all their saliva for 3 h immediately following beetroot juice ingestion completely blocked the rise in plasma nitrite and

the reduction BP. This increase in plasma nitrite is also inhibited by the use of an antibacterial mouthwash just prior to nitrate ingestion in humans Mirco Govoni et al. 2008.

Many of the biological effects seen with nitrate and nitrite are associated with formation of NO and NO-related substances. However, it is presently evident that nitrite itself mediates biological effects. Additionally a variety of different nitrogen oxides can be formed in the acidic gastric milieu and elsewhere which can mediate NO-like signalling. Thiol groups (-SH) in cysteine residues of proteins might undergo S-nitrosation by nitrite, generating S-nitrosothiols which can act as NO donors Stamler, Jonathan S. et al. 2001 or generate post-translational modifications that can alter protein function. Other responses might be mediated by the generation of electrophilic nitro-fatty acids (NO₂-FA). Nitration of unsaturated fatty acids can be induced by nitrite-derived nitrogen oxides (NO_x), especially the nitrogen dioxide radical (\cdot NO₂), formed in high amounts during gastric acidification of nitrite. The electrophilic properties of nitrated fatty acids can mediate anti-inflammatory signalling through activation of nucleophilic proteins such as the peroxisome proliferator activated receptor- γ (PPAR- γ) and suppress inflammatory NF- κ B signalling. Although endogenous formation of nitro-fatty acids occurs, the levels found in plasma are in a low range, and some authors have questioned if they play a role in normal physiology H Rubbo 2013.

Importance of keeping the oral microbiota healthy

The extensive literature on NO₃-NO₂-NO largely leaves the oral microbiota subject matter untouched. The so called "responders" and "non-responders" might be due to precisely the degree of appropriate oral care that each individual practices. Emerging evidence from the gastro-intestinal tract, genito-urinary tract and respiratory indicates that there are several health benefits to hosting a complex and diverse microbial community.

HOW TO USE