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# Nitric Oxide In Health And Diseases

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A B S T R A C T

Nitric Oxide (NO) is color less, readily diffusible, highly reactive lipophilic and chemically unstable Free radical gas. NO is formed form arginine by enzyme nitric Oxide synthase. No acts in a paracrine or even autocrine manner. It causes asodilatation, act as neurotransmitter, Prevent Platelet aggregation and also play important role in macro-phase function. Deficiency leads to, increase vascular tone, reduced thrombolitic activity. Use of NO in pediatric pulmonary hypertension and in sexual dysfunction is increases day by day. The discovery of NO as an essential effectors of many Biological phenomenon surely stand out as one of most important achievement of 20th century of Biomedical investigation.

Key Words: Nitric oxide, NOS, Relaxation

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## Introduction

Nitric Oxide is color less, readily diffusible, highly reactive lipophilic and chemically unstable Free radical gas. It is simple, hetrodiatomic molecule with broad and diverse effect on human biology by acting as Mediator in much reaction. NO˙ is produced by many cell type, and causes vasodilatation, act as neurotransmitter, Prevent Platelet aggregation and also play important role in macro- phase function[1].

Nitrite (NO2 –) and nitrate (NO3 –) are known predominantly as undesired residues in the food chain with potentially carcinogenic effects[2,3] or as inert oxidative end products of endogenous nitric oxide (NO) metabolism. However, from research performed over the past decade, it is now apparent that nitrate and nitrite are physiologically recycled in blood and tissues to form NO and other bioactive nitrogen oxides [4-7].

Lauder Branton is the person to use nitric oxide as therapeutic agent for treatment of angina pectoris in the form of nitroglycerin and amyl nitrites. In 1977 Ferid Marad showed that the vasodilator effect of nitroglycerine is due to release of NO. In 1980 Robert furchgett showed that endothelium derived relaxing factor is required for arteriolar dilatation. In 1987 Louis Ignalro showed that Endothelium Derived growth Factor is chemically NO. For above work they got noble prize in 1998 [8-9].

Nitrite reduction to NO and NO modified proteins during physiological and pathological hypoxia appear to contribute to physiological hypoxic signaling, vasodilatation, modulation of cellular respiration and the cellular response to Ischemic stress[7, 10, 11–16]. This article reviews the metabolism and biological roles of NO within the body and its potential therapeutic use.

**Chemistry:** Nitrite is unique to the nitrogen oxides in its redox position between oxidative (NO2 radical) and reductive (NO radical) signaling and its relative stability in blood and tissue[17]. Once nitrite is formed, there are numerous pathways in the body for its further reduction to NO, involving haemoglobin[7, 18], myoglobin [18, 19], xanthine oxidoreductase[20-22], ascorbate16, polyphenols [23, 24] and protons [4, 5]

Nitric Oxide is an uncharged molecule having an unpaired electron, so it is highly reactive free radical. So It is correctly written with superscript dot NO = NO*.Being lipid soluble it can freely diffuse across the membrane. Half- life is very short, only about 0.1

second, than it will convert by Oxygen and water into nitrate and nitrites.

**Synthesis:** Reduction of nitrite to NO occurs in blood and tissues and proceeds through several enzymatic and non-enzymatic pathways. The acidic reduction of nitrite results in the generation of NO.

In 1994, two groups independently described intragastric NO generation from salivary nitrite in humans [4, 5]. This process does not require NOS activity, but instead involves the entero-salivary circulation of inorganic nitrate. Dietary nitrate is rapidly absorbed in the upper gastrointestinal tract. In the blood, it mixes with the nitrate formed from the oxidation of endogenous NO produced by NOS enzymes form arginine.

NOS is dimeric protein; it contains Heme, FAD, and FAM Calmodulin molecule tetrohydro Biopterin.

BH4: TetrahydroBiopterin co – factor required for overall reaction. It is factor by which oxygenation of NO or Citruline take place.

Heme: Iron Protoporphyrine is functional prosthetic group associated with all three isoform of nitric oxide synthase and important for interaction Between Flavoprotein and Heme Protein.

4-aminoacid motifs: Glycine – lucine, Glycine-Phenylanalin. Function is not clear but

may serve to target protein to specify site in cell.

NADPH: Electrone donor.

FAD, FMN: Use for flow of electrone

Calmoduline: a 14 KDa $Ca^{+2}$ Binding protein is involved in the control of electrone flow. Between prosthetic group in oxygenase molecule.

*There are three isoform of Nitric Oxide synthese.*

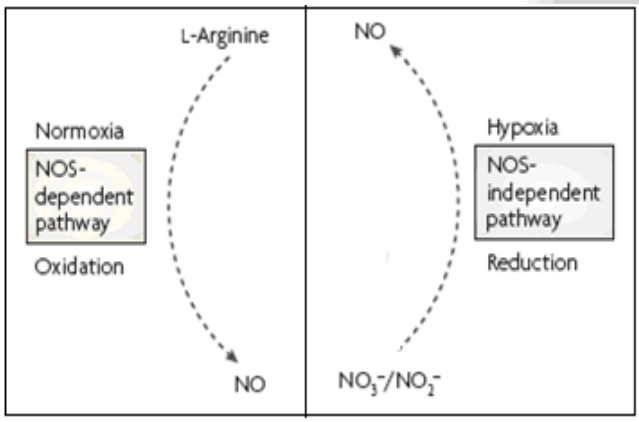
NOS I or n NOS or Neuronal NOS: it is Seen in central and peripheral nerve, in cerebellum and Gastro Intestinal tract. It is Mainly Cytoplasmic enzyme and Gene located on chromosome 12. If knocked out in the rat result in the vascular stroke, aggressive sexual behavior in the male rate

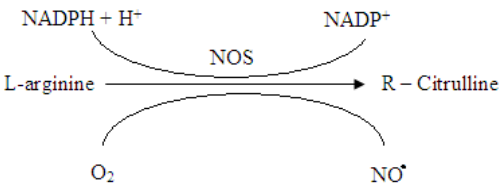
NOS II or i NOS or inducible NOS: Present in monocytes, macrophage, smooth muscle cells microtubule, endothelial cells, fibroblast cardiomyocyte, hepatocytes and megakeryocite. It is induced by cytokine (IC – 1) during inflammation. It is also Cytoplasmic enzyme but Gene located on Chromosome: 17 and If knocked out, more susceptible to certain type of infection.

NOS 3 or e NOS or endothelial NOS: Seen in endothelial cells Platelet, endocardium and myocardium and at plasma membrane. Gene for this type of NOS is located on Chromosome 7 and If knocked out, result in increase blood pressure.

There are two parallel pathways for the generation of bioactive NO in mammals. NO is a key signaling molecule that serves to regulate a wide range of physiological functions. It is classically produced from l-arginine and oxygen by a family of enzymes, the NO synthase (NOSs). In other pathway, the inorganic anions nitrate and nitrite are reduced to form bioactive NO in blood and tissues during physiological hypoxia.

Although NO generation by NOS becomes limited as oxygen levels fall, the nitrate–nitrite–NO pathway is enhanced. By the parallel action of both of these pathways, sufficient NO generation is ensured along the physiological and pathological oxygen and proton gradients.



Sequence of event during NO synthesis is as Follow:

Enzyme utilize NADPH and molecule of O2 > From molecular oxygen, one is added to NO and other into Citruline > The qunidionitrogen of arginine is incorporated into NO > NADPH donates two electrone to FAD which in tern reduce to FMN > Flavin than reduced the heme iron prosthetic group $Fe^{+2}$ to which oxygen can now bind for the oxygenation of the substrate L – arginine.

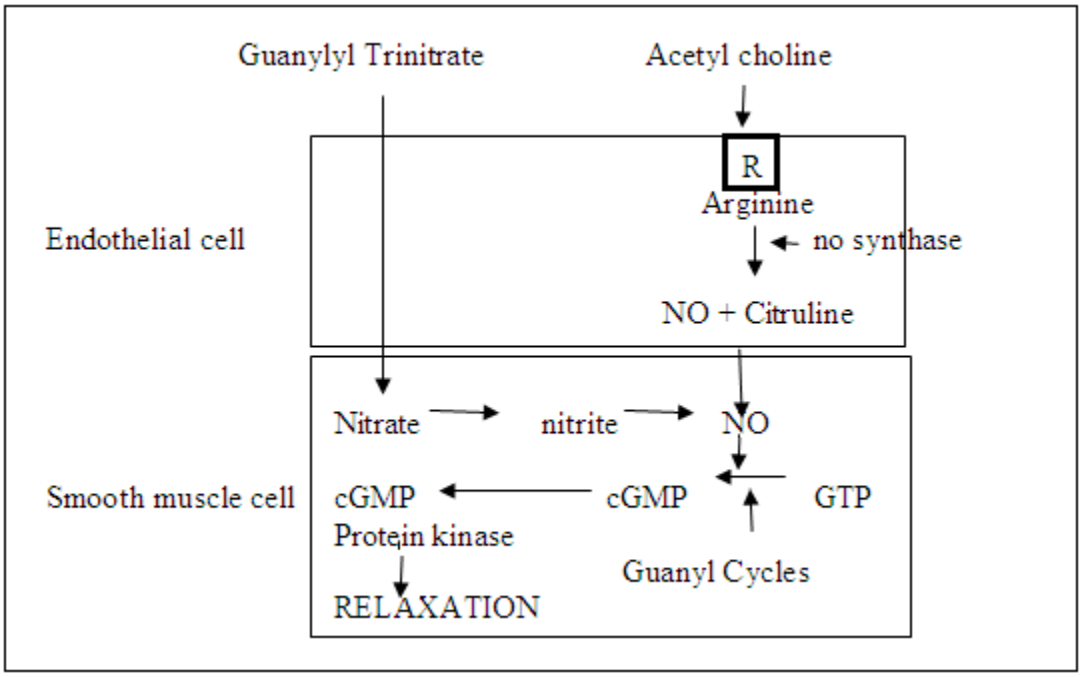
Enzyme activity is totally depend on bound calmoduline which realize high concentration of $Ca^{+2}$ for N and E isoform of NOS.The reaction is totally inhibited by carbon monoxide.

This rate of NO formation increases as hemoglobin oxygen saturation decreases, suggests a hypoxia-regulated mechanism of nitrite bioactivation. These chemical properties, and supporting physiological studies, suggest that hemoglobin may function as an allosterically regulated nitrite reductase that may contribute to hypoxic signaling and hypoxic vasodilation[7,11,25,26]. Interestingly, recent studies suggest that NO formed from nitrite reduction (equation 1) can react with a second nitrite that is bound to methaemoglobin (HbFe3+) [27].

The formation of bioactive nitric oxide (NO) from the inorganic anion nitrite is generally enhanced under acidic and reducing conditions. In the acidic gastric lumen, NO is generated non-enzymatically from nitrite in saliva after formation of nitrous acid (HNO2) and then decomposition into NO and other reactive nitrogen oxides. This NO helps to kill pathogenic bacteria and it also stimulates mucosal blood flow and mucus generation, thereby enhancing gastric protection.

**Mechanism Of Action:** No acts in a paracrine or even autocrine manner. Signaling function of No begin with its binding to protein receptor on or in the cell. The binding site can be either A normal iron in the protein or One of its S atomic e.g. cysteine

In either case binding trigger an allosteric change in protein, which in turn triggers the formation of a second messenger within the cell. Most common target is Guanylyl Cyclase




**Antagonist and inhibitors of NOS:** N mono –methyl arginine competitively inhibit it so NOs used in septic shock to Prevent Hypo tension. Asymmetric dimethyl arginine an endogenous analogue acts as NOS inhibiter which is increased in homocystenemia only pre - eclampsia which isone of the factor leads to decrease NO production which leads to Hypertension during Eclempsia.

Glucocorticoids also inhibit NOS transcription which may be account for part of their protective effect is septic shock syndrome. In case of loss of Bioavailability treatment is Dilatory supplementation with L arginine anti oxidant therapy which improved the functional deficit of NO. Arginine is present in

relatively Large amount in nuts (Brazil nuts and almonds), Shell fish, Meats including Beef.

**Metabolic fate of NO:** NO combine with $O_2$ to Form NO2 These nitrites are excreted through urine. No combine with hemoglobin it is converted to NO3 and nitrites, excreted in urine. Very low amount is expelled via lung. On exposure to super oxide anion (O2 -) nitric oxide is converted to highly reactive free radical.

Proxy nitrite (OONO) which cause lipid par oxidation, cell injury and cell death. Thus as a free radical , NO readily undergo addition, substitution redox and chain terminating reaction which serve as molecular basis of its biological effect.

**Measurement of NO:** NO synthase requires NADPH and it is NADPH diaphoreses for which histochemical stain has been available. Thus, it is easy to stain for NO synthese in Brain and other tissue. The level of metabolic product (nitrines, nitrate, 3 nitro tyrosine in blood and urine are marker of NO production.

**Physiological Action Of Nitric Oxide**

On Blood vessels[9]: No is potent vasodilator. The normal pressure is maintained by No liberated by endothelial NOS. It is observed that when various derivative of Arginine that inhibit No synthesis are administered to experimental animal there is prompt rise in Blood pressure which suggest that tonic release of NO is necessary to maintain normal blood pressure.

At each systole, the endothelial cells that line the blood vessels release a puff of NO. This diffuses into the underlying smooth muscles cells causing them to Relax. And thus permit the surge of blood to pass through easily. Mice whose genus for the No synthese found in endothelial cell (eNOS) has been "knocked out "suffer from hypertension.

Mechanical shares stress on vessel wall the important stimuli to induce No Synthesis. No causes cerebral, coronary, renal and muscle arteries to dilate. nNOS in neuronal and glial cells contributes to regulation of cerebrovasculer tone also help in memory and learning through its involvement in long term potentation in central nervous system.

Vasodilator action of NO is proved by various studies i.e. humans breathing NO gas exhibit increases in peripheral forearm blood flow that is associated with increases in plasma nitrite59, suggesting that nitrite could be a stable endocrine carrier and transducer of NO‐like bioactivity within the circulation60. More recent dose-response experiments in normal human volunteers reveal significant vasodilation of the forearm circulation already at concentrations as low as 300 nM61 and a significant decrease in blood pressure after nitrate ingestion, associated with an increase in plasma nitrite levels from 140–220 nM62.

On Platelet: Basal production of No by eNOS inhibit both adhesion and aggregation of platelet in vasculatures.

On intestinal smooth muscle: NO is no adrenergic and non – cholinergic neurotransmitter especially in GIT and urinary tract. No relaxes gastrointestinal smooth muscle and leads to reduced mobility, relaxation of sphincter of odd, and relaxation of lower esophageal sphincter. A deficiency of NO producing neuron with decreased mobility is responsible for infantile hypertrophic pyloric stenosis and HIRSPRUNG DISEASE. Another proposed physiological role for gastric NO is in the regulation of mucosal blood flow and mucus generation[28] A role for salivary nitrite in regulating gastric gastrin release has also been suggested[29]

On Bronchial smooth muscle: endogenously produced NO may contribute to maintenance of Basal, Basal Bronchial and basal Pulmonary arterial wall tone.

Role in Reproductive system[30]: In Penile erection: The erection of penis during sexual excitation is mediated by No. Released from nerve ending close the blood vessels of Penis, Relaxation of these muscle cause blood to pool in blood sinuses producing an erection

Role in fertilization: The acrosome at the tip of sperm head activates its NOS when it enters the egg. The resulting release of No in egg leads to (i) blocking the entry of additional sperm and (ii) orienting pronucles for fusion.

Role in Pregnancy: NO inhibit uterine muscle contraction during pregnancy. Also help in cervical Ripening.

Role in endocrine system: Stimulate GnRh secretion from Hypothelemus, exocrine pancreas secretion adrenaline from Adrenal medulla.

Role in Kidney: NO increases tubular blood flow and which leads to increases GFR and second mechanism for increase in GFR is by inhibition of NO.

NO and immunity: Production of NO by i NOS in macrophages, lymphocyte and neutrophil is an important determinant of immune and inflammatory response. NO is hence play role in secretary immunity. No help in non specific immunity mile with "knocked

out "gene for NO suffer from severe infections diseases.

While the scientific community had focused on the potentially harmful effects of nitrate and nitrite1, the well-known antibacterial effects of NO [31-34] suggested a role for gastric NO in host defense [4, 5] interestingly; enteropathogens can survive for a surprisingly long time in acid alone, but the combination of acid and nitrite results in effective killing 4. NO and other reactive nitrogen oxides formed from acidified nitrite act on multiple bacterial targets including DNA, proteins and components of the cell wall [38,47].

NO and nervous system: NO and ANS: Non cholinergic / Non adrenergic transmitter. e.g. in penis and Intestine. NO and medulla Oblongata : $O_2$ deficiency activate, NO sensitive cell which in tern activate respiratory center which increases rate and depth of Breadth NO and cerebral center : Highly diffusible so magnifies localize action and help in memory and long term potentiation.

**Pathological Effect**

Deficiency of NO : It is associated with Hypertension as illustrated by recent observation in marine model of $NOS_3$, in which gone inactivation done by homologues recombination. Deficiency represents either a true deficiency of molecule or inactivation of it by reactive oxygen derived free radical. This deficiency leads to

- ✓ increase vascular tone
- ✓ reduced antithrombolitic activity
- ✓ increase endothelial prolipheretive activity
- ✓ increase endothelial permeability
- ✓ enhance susceptibility to low-density lipoprotein to oxidation.

Deng and Rapp have demonstrated a clear linkage Between the $NOS_2$ gene locus and self sensitive hypertension in Dahl S rat. In chronic renal failure: The concentration of dimethyl arginine, a naturally occurring derivative of L - Arginine is increased and competitively inhibit nitric oxide syntheses activity, possible contributing to hypertension of chronic renal failure.

In Bacterial sepsis induction of NOS2 occur and the elaboration of NO in this setting accounts for the hypo tension and septic shock state and contribute to the hemodiathesis of sepsis by profoundly inhibiting platelet function. The myocardial depression associated with septic shock may be explained in part by the inhibition of myocardial contractility by NO.

Ischemia Reperfusion Injury : may contribute to elaboration of NO through increased expression of iNOS. and accompanying Myocardial depression that follows successful coronary thrombolysis or revascularization.

In Laennec's cirrhosis: Increased No production owing to the induction of $NOS_2$ in hepatocytes, Fibroblast and endothelia cell is believed to responsible for the hyperdynemic circulatory shock. NO Inhibit protein synthesis in the hepatocytes at post transnational level and also inhibit several mitochondrial enzyme involved in electron transport including Cis – aconitose.

Inflammation: No enhances the effect of cyclooxygenease and stimulate the production of Pro-inflammators. No production can be induced through up gradation of i NOS . by a number of factor involved in inflammation e.g. IL, Interferon's, gama, TNF- □□and etc.

Excessive amount of NO impair myline production (Hall mark feature of multiple sclerosis ) In Joint tissue it result in acute and chronic inflammation, characteristic sign of arthritis.

**Therapetic Use Of No :**

Use of Nitric is not totally new science inhaled amyl nitrite was used to treat asthma in 1866. after that Numerous studies have now confirmed the vasodilating effects of low-dose nitrite in mice, rats, sheep, dogs, primates and humans [35-41].

*Use of NO in persistent Pulmonary HT :* In sheep models of PPHN, inhaled nitrite was converted to NO gas in the lung and selectively dilate the pulmonary circulation78. In such diseases, which are characterized by regional ischemia and vasoconstriction, nitrite may provide an ideal stable and naturally occurring therapeutic NO donor. NO is vasodilator of choice in term babies with Persistent Pulmonary Hypertension. NO significantly improve Oxygenation. The therapeutic role of exogenously administered nitric oxide is based on its direct vasodilator activity and extremely short life time. The inspired concentration of nitric Oxide may be monitored by chemilumescent detector.

Clinically useful Bronchodilatation may be produced by inhaling low concentration ( 60 ppb to 60pp ) for selective Pulmonary vasodilatation and improvement in oxygenation. (Selectivity for Pulmonary circulation is due to the inhaled route of administration and its rapid deactivation by hemoglobin in pulmonary blood so that vasoactive concentration does not reach systemic circulation...)

*Sindenafil citrate :* inhibits production of Phosphodiesterase enzyme which Break down nitric oxide . So this drug increase level of NO which relives smooth muscle in corpus cavernosa and increase blood flow into penis and make it erectile. Other vasodilator agents used are

- ✓ Nitroglycerine - Use in angina pectoris
- ✓ Sodium Nitropruside - Hypertensive crisis
- ✓ S- nitriglutathions - inhibit Platelet aggregation

*Tissue protection in ischemia–reperfusion injury. :* Systemic NOS-independent NO formation from nitrite was first demonstrated in the ischemic heart[6]. Physiological and therapeutic levels of nitrite exert potent cytoprotection after prolonged ischaemia and blood-flow reperfusion in liver[12,42], heart [12,43], brain[44] and kidney[45]. These findings suggest an opportunity for nitrite therapy for human diseases associated with ischaemia–reperfusion, such as myocardial infarction, stroke, solid-organ transplantation, cardiopulmonary arrest and sickle-cell disease

Dietary nitrate increased gastric NO levels and potently protected against the macroscopic injury caused by NSAID exposure. Nitrate pretreatment decreased mucosal myeloperoxidase activity and expression of iNOS, which is indicative of reduced tissue inflammation. The protection afforded by nitrate probably relates to increased gastric mucosal blood flow and mucusgeneration and reduced epithelial permeability [46,47]. An additional protective effect of nitrate on ulcer development may occur through inhibition of *Helicobacter pylori*[48].

*Topical administration of acidified inorganic nitrite.:* An inorganic nitrite salt such as sodium nitrite (NaNO2) is combined with an acidifying agent (for example, ascorbic acid). This mixture rapidly releases NO and other nitrogen oxides and has been evaluated for its antimicrobial activity. Topical application of acidified nitrite to the skin has proved effective in various skin infections[49-51]

*Role of NO in tumor cells*[52] is by Direct and Indirect damage. It Directly damage to DNA by Inhibition of DNA synthesis and inhibition of rate limiting Enzyme reboneucleotide Reductase. Reduced activity of Cis aconitase and loss of large fraction of the iron Pool, have also been suggested as possible mechanism. NO can affect mitochondria leading to reduction of consumption and damage to Complex I and II in mitochondrial electron transport chain, it also leads to reversible inhibition of Complex IV and induction of apoptosis.

Low dose and short duration of treatment suggest that the risk of any carcinogenic effects is negligible. In fact as stated above, a large consumption of nitrate-containing vegetables may provide similar or even greater systemic loads of both nitrate and nitrite. If nitrite is to be used in much higher doses over prolonged periods of time, this issue will naturally have to be addressed.

*USE OF NO BY OTHER ANIMAL AND PLANTS*

Fire Fly's use NO to turn on their flashes. Plant also employ NO as weapon against invading pathogen. Infection of plant triggers the formation of NOs that like the animal version make NO from arginine. Release of NO by the infected cell triggers number of defense responses.

## FUTURE OF NO

NOS gene. Therapy : Gene therapy with NOs3 and use of long acting NO donor limit vascular smooth muscle proliferation, following denuding endothelia injury, which suggest potentially useful therapy for limiting re stenosis following angioplasty

(2) Nitric oxide synthese is induced in the joints of patient with Rheumatoid arthritis, in the gut of patient with ulcerative colitis and the ventricle of patient with cardiomyopathy.

(3) Exhaled NO is a novel of biometer of respiratory health in epidemiological studies.

Future clinical studies will elucidate whether nitrate can offer a nutritional approach to the prevention and treatment of disease. If such investigations point towards a protective effect of nitrate, the current strictly regulated levels of nitrate in food and drinking water may need to be reconsidered. Thus, The discovery of NO as an essential effectors of many Biological phenomenon surely stand out as one of most important achievement of 20th century of Biomedical investigation.

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