

# Vascular pulsations stimulating nitric oxide release during cyclic exercise may benefit health: A molecular approach (Review)

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Received October 25, 2000; Accepted November 23, 2000

**Abstract.** It is widely assumed that all exercise, regardless of the degree of difficulty or strenuousness, is good (no pain-no gain). In this speculative review of the literature and our research findings we highlight the fact that strenuous exercise taken to the extreme initiates an immune and vascular pro-inflammatory situation. However, mild cyclic exercise appears to produce health benefits for an individual. In part, this is due to vascular cyclic pulsations, occurring in mild exercise, stimulating constitutive nitric oxide synthase derived nitric oxide release. This in turn down-regulates vascular endothelial cells and immunocytes, as well as their interaction and inhibits the disassociation of NF- $\kappa$ B, preventing the production of proinflammatory cytokines. The nitric oxide so generated may even scavenge excess free radicals, preventing tissue damage. Prolonged strenuous exercise appears to limit these positive phenomena because of the maintained and prolonged high blood pressure that reduces the cyclic pulsations, limiting nitric oxide production. We further note that pathological conditions, i.e., Parkinson's disorder, may benefit from mild exercise, i.e., cyclic nitric oxide production, since the inactivity associated with this disease may lead to compromised nitric oxide production, initiating a progressive deterioration of tissues, including peripheral adrenergic neurons, due to a lack of adequate basal nitric oxide levels required to maintain the vascular microenvironment in a mild state of inhibition. We conclude that mild exercise represents an alternate and economical therapy to preserve health and/or diminish the rate of decline of the normal physiological processes that may even be associated with aging.

## Contents

1. Introduction
2. Nitric oxide
3. Exercise
4. Pathology
5. Conclusion

## 1. Introduction

Exercise, in the general sense, has always been associated with health. In this regard, the healthy end product has been deduced by enhanced cardiovascular performance, increased muscle strength, a feeling of greater energy and enhanced muscle tone and mass to name a few factors. However, is strenuous exercise the best form of this activity, i.e., no pain-no gain? Strenuous exercise can be regarded as physical stress (1). Many clinical physical stressors (e.g., surgery, trauma, burn, and sepsis) can and do induce hormonal and immunological responses with similarities to strenuous exercise (1). Recent experiments have questioned this aspect of 'conventional' wisdom concerning exercise, in that more moderate forms of exercise have been found to be highly beneficial. Given this, it becomes important to determine exactly what exercise is expected to accomplish and how this complements the physical capabilities of an animal, i.e., man. After all, the neural, cardiovascular and muscle systems have taken millions of years to evolve before cognition clouded their purpose. That is, these systems were designed to gather food and provide for mobility. In this regard, the complementary systems, i.e., immune, digestive etc., can be considered to protect an animal and provide for longevity.

In regard to exercise, a recent study demonstrated that neutrophils became mobilized and activated in response to exercise (2). Furthermore, in the recovery phase after intense exercise the following was found: i) suppressed natural immunity of blood lymphocytes; ii) decreased concentration of secretory IgA in mucosa; iii) increased blood concentration of neutrophils; and iv) increased levels in the blood of inflammatory cytokines (1,3). Thus, after intense long-term exercise, the immune system can be characterized as being in

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*Key words:* nitric oxide, endothelium, norepinephrine, exercise, NF- $\kappa$ B, Parkinson's disease, aging, vascular tissue

an activated proinflammatory state [e.g., interleukin (IL)-1, 6 and TNF], resulting in a suppression of the cellular immune system (3). It has been demonstrated that a close association exists between muscle damage and increased levels of interleukin-6 (3), supporting the view that the immune system may, for the short-term, be hyperactivated and thus, dysfunctional.

Interestingly, the diffuse inflammatory response associated with major surgery, i.e., coronary artery bypass grafting, is also characterized by the increased presence of activated granulocytes, proinflammatory cytokine induction and cellular immunosuppression via hyperactivation of the immune and vascular systems (4-10). Based on this comparison, it may be safe to conclude that strenuous exercise may not be the best thing for individuals, especially those just attempting to generally enhance their physical activity. In this regard, we surmise that processes exist to restore the 'body' to its physical resting condition since continued immune and vascular dysfunction would lead to death. We also speculate that given the degree of this self-induced trauma whether surgery or strenuous physical exercise, the resting/restorative/relaxing phase of exercise, counter-intuitively, is the most important phase of exercise. The restorative/recovery biochemical and physiological processes enable cellular communication to re-establish itself, limit excitation and promote healing. Thus, these less well-examined processes establish the baseline of normal health since they maintain the organism in this state most of the time within a 24-h period.

In this speculative review we will discuss various cellular processes that probably participate in maintaining and establishing homeostasis following exercise. It is important to note at the onset of this discussion that the processes we will highlight are of a small nature when considered individually. They function locally. However, their impact is physiologically enormous because they occur throughout the body: their significance summates exponentially. In plant transpiration, the movement of water up the plants vascular tissue occurs because of evaporation of single water molecules at the plant leaf. However, when considering all the leaves and pores on each leaf, this movement, due to the weak hydrogen bonds of juxtaposed water molecules, becomes a formidable force that can raise water up a 300 foot redwood tree. Here we see how weak forces can combine exponentially to provide a large movement/force. In this regard, we surmise small physiological processes before, during and after exercise are working in a similar manner. We will then attempt to relate these processes to various pathological conditions where their combined activity may have a substantial therapeutic value. Thus, we will also advance the hypothesis that mild cyclic exercise works via the enhancement of these normally small stabilizing factors since they respond to internal rhythmic body signals, i.e., vascular pulsations.

## 2. Nitric oxide

Nitric oxide (NO) is a major gaseous signaling molecule in several systems, including the immune, cardiovascular and nervous systems (10-15). NO is produced from L-arginine by the enzyme NO synthase (NOS) (14,16), occurring in three isoforms: endothelial (e), neuronal (n) and inducible (i) NOS.

The first two are constitutively expressed and  $\text{Ca}^{2+}$  dependent, whereas inducible (i) NOS is  $\text{Ca}^{2+}$  independent. The three forms of NOS are encoded on three distinct genes on chromosomes 7, 12 and 17, respectively (11-13,17). The presence of constitutive and inducible forms of NOS suggests that they may have distinct functions or the same function enhanced (18). In a previous report, we have developed the hypothesis that cNOS is responsible for a basal or 'tonal' level of NO that keeps particular types of cells in a state of inhibition and that activation of these cells occurs through disinhibition, i.e., diminishing cNOS derived NO levels (18).

Concerning cNOS, it can be stimulated to enhanced the release of cNOS derived NO for short durations of time by way of various signaling molecules, i.e., estrogen, anandamide, morphine etc. (18). This enhanced NO release can have profound physiological actions, which are evident long after NO has returned to its basal level (19,20). For example, endothelial cells briefly exposed to morphine and eNOS derived NO change their shape from elongated to round, a process that takes several hours (19).

iNOS is induced by various signal molecules, e.g., pro-inflammatory cytokines (14,21,22). The induction of iNOS is usually seen after a 3-4 h delay; iNOS is capable of producing NO for 24-48 h (21,22). These data suggest first, that NO is always present and, second, that the levels of NO can be regulated either rapidly or slowly depending on the organism's needs (18). The presence of different regulatory processes imply that NO has different functions and/or that NO stimulated function can occur by degrees, that the levels of NO must be progressively increased in order for it to exert its function, or some combination of these phenomena. Additionally, it is important to note that NO functions as a vascular, immune and neural signal molecule and also has general anti-bacterial, anti-viral actions and the ability to down-regulate proinflammatory events (22-29). We have hypothesized that certain classes of cells are always 'on', i.e., respond to environmental changes, and that this low level of NO which is cNOS derived, provides an organism with a major pathway that functions to dampen microenvironmental 'noise' which would otherwise non-specifically and inappropriately activate them (18). NO may control the threshold for activation of these cells. This kind of activation really represents a disinhibition process (as noted earlier), i.e., an overcoming of the inhibitory influence of NO by changing the balance between basal NO and the levels of excitatory signals (18). For example, when excitatory signals, i.e., interleukin-1, are present at detectable levels the tonal inhibition due to NO is overcome and activation occurs.

*Endothelial constitutive nitric oxide.* Since endothelial cells express cNOS-derived NO, we surmise that endothelial cells are always 'on', that is, they respond to environmental changes by overcoming the inhibitory balance of low basal NO (18,23). This is also noted since eNOS derived NO in human blood vessels, diminishes the adherence of monocytes and granulocytes to the vascular endothelium (18,23) and induces endothelial rounding (18,23). This process can down-regulate pro-inflammatory events (18,23). It helps maintain blood fluidity (30). This balance of 'blood' NO levels may also be modulated by hemoglobin, a NO scavenger (18). These studies suggest

## Mechano-transduction

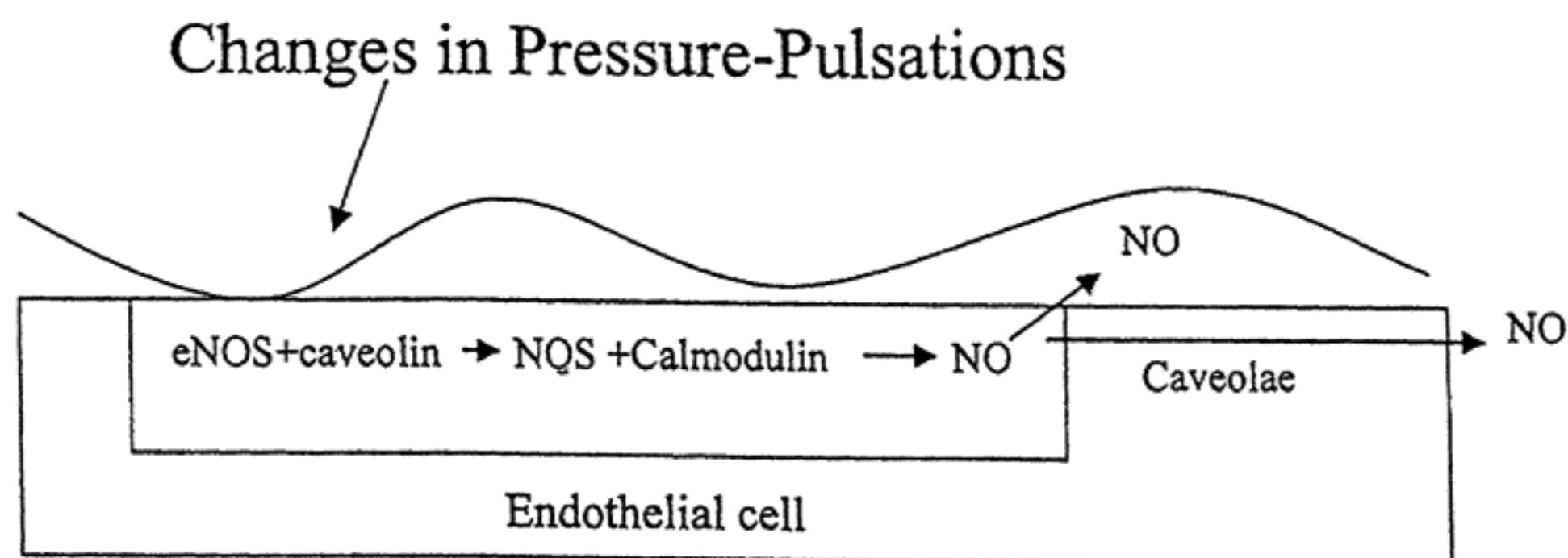


Figure 1. Acute changes in pressure (pulsations) or shear stress stimulate a rapid release of nitric oxide (NO) via eNOS activation. eNOS is enzymatically active in the caveolae. Pressure and/or flow *in situ* rapidly activate eNOS by inducing its dissociation from caveolin, the inhibitory protein, and coupling to calmodulin. This links mechano-transduction and eNOS activity. Thus, the pulsatile flow of blood in microvessels may be essential for their proper functioning, including a modest form of this phenomenon in capillary beds. We surmise that cNOS contributes to the pulsatile release of NO.

that capillary endothelial cell function and release of NO plays a critical role in the hemodynamic, humoral and inflammatory signals to which it is constantly exposed by circulating blood cells. For these reasons, endothelial cell dysfunction, induced for example, by the harmful accumulation of other free radicals in endothelial cell lining, can interfere with NO release and inhibit vasodilation (31,32). Consequently, impaired NO homeostasis may promote endothelial cell damage and expose end organs to assorted vasculopathy (13,33).

Recently, we have demonstrated (10,19,34) estrogen can also act via eNOS derived NO to affect cell shape (35-37). Acting via a cell surface receptor, estrogen stimulates eNOS to release NO; this initiates down-regulation, characterized by cell rounding and inactivity. Furthermore, once the effect of eNOS derived NO activity (round and inactive) wears off, the cells, for a short period of time, exhibit a simple rebound effect characterized by hyperactivity (18,20,23). This demonstrates that NO, under some circumstances, can appear to activate cells, however, this may really be occurring via a rebound from inhibition.

Thus, at different times, NO can promote cell shape changes that may represent an activated (mobile) or inactivated (round) cell state. Low, basal levels of NO, may exert functionally significant constraints on cell shape. The result of this phenomenon, we propose, is that NO keeps endothelia stationary and maintains their shape and activation state in the microvasculature and that an absence of basal NO results in cell shape distortions (18,23).

Recent studies have enabled our understanding of NO intracellular mechanisms that are in line with the external cell shape changes. NO can cause cells to extend and retract portions of their cytoplasm (38-40). This action may be effected via ADP-ribosylation of actin causing actin depolymerization resulting in the inhibition of shape changes by these cells (20,41,42).

It would appear then, that endothelial eNOS activity is vital for the stabilization of the vascular microenvironment and that this stabilization occurs, in part, by regulating cell

shape. Here the level of basal NO appears to be a key factor in this regulatory process and the capillary bed is especially prone to this regulation because it is basically composed of endothelia. We surmise, a lack of proper functioning within the vasculature may have significant pathogenesis impact in various organ systems (43).

*Vascular pulsations.* We must ask the question, what regulates basal NO? In part we believe the answer lies in the arterial vascular pressure pulses originating in the heart and the passive pressure inducers in the venous system, which propagate through the vasculature. In a recent study, we demonstrated that sustained high pressure stimulated NO release from intact human saphenous vein pieces at progressively smaller levels, demonstrating an impairment (44). Additionally, pulsatile flow at low pressure maintained an even release of NO, demonstrating the significance of normal vascular response to pressure and NO processes. eNOS has also been shown to be important for the regulation of basal blood pressure, and abnormal basal NO production has been described in unrelated essential hypertension (45), further demonstrating NO's significance (18,43).

We found that impaired NO release, maintaining human vessels at high pressure, resulted in a significantly higher level of granulocyte adherence in blood vessels, thus mimicking a proinflammatory state (44). The mechanism underlying this process has recently been explored (Fig. 1). Rizzo and colleagues (46), besides demonstrating that acute changes in pressure or shear stress stimulate a rapid release of NO via eNOS activation, using subcellular fractionation of the rat endothelial membranes and their caveolae (small invaginations in the plasma membrane of endothelial cells involved in cell signaling), demonstrated that eNOS is enzymatically active in the caveolae (Fig. 1). Thus, pressure and/or flow *in situ* rapidly activates eNOS by inducing its dissociation from caveolin, the inhibitory protein, and coupling to calmodulin. These data link mechano-transduction and eNOS activity, directly supporting the present hypothesis. Sustained high

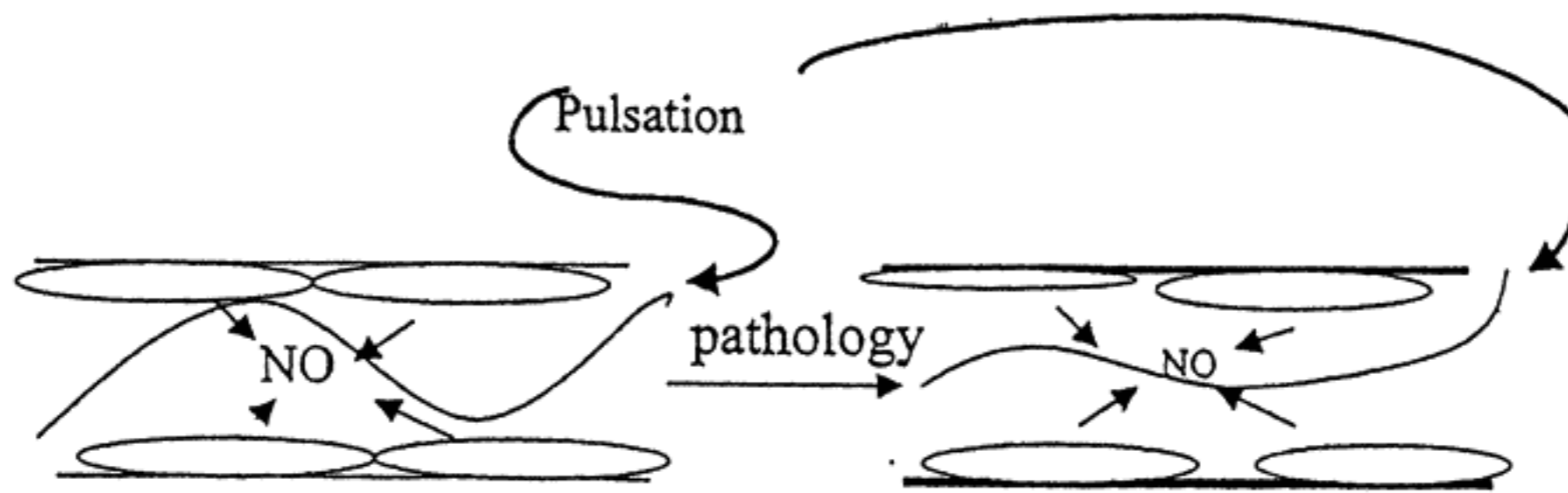


Figure 2. Illustration of normal pulsatile release of NO that serves to maintain endothelial cell shape (left). We surmise that factors leading to a diminishing of the dynamic range of the pulsations, i.e., hypo- or hypertension, diminish cNOS associated NO release (right) since the blood-pulsations are compromised. This in turn alters endothelial shape, creating gaps between the cells that may lead to endothelial thickening, diminishing energy metabolism and inducing the formation of free radicals (see text). We speculate that mild exercise can restore the condition represented on the left by re-establishing NO release by reintroducing pulsatile pressure flow where it once was reduced due to inactivity, i.e., as in Alzheimer's disease, Parkinson's and to an extent, normal aging.

pressure or low pressure may uncouple this process and chronic hypertension/hypotension could greatly diminish this action with time (44) (Fig. 2). Thus, the pulsatile flow of blood in microvessels may be essential for their proper functioning, including a modest form of this phenomenon in capillary beds.

Furthermore, endothelia, from non-insulin dependent severe diabetics do not exhibit a tonal level of NO (Bilfinger TV and Stefano GB, unpublished data) and in these individuals vascular disease causes disability and death (47). A number of authors, including our group, have attributed vascular disease, in part, to alterations associated with eNOS derived NO. Moreover, because this process represents such a low level of NO release, it may take many years to manifest itself into a symptomatic disorder. The reason for this slow vasculopathic evolution may be partly because during the initial lowering/diminishing of eNOS derived NO, eNOS can be stimulated by other signaling molecules (e.g., estrogen, anandamide, interleukin-10, acetylcholine, bradykinin and morphine to name a few), providing for a limited short-term presence of NO, compensating for limited basal NO (18,23). Indeed, this progressively debilitating endothelial scenario is in all probability associated with normal vascular aging.

As suggested by other recent studies, pulsatile endothelial NO release may also be generated from cyclic agonist-promoted increases in  $[Ca^{2+}]_i$  that induces the dissociation of the calveolin-eNOS complex, leading to enzyme activation (48-51) (Fig. 1). In this regard, prolonged agonist stimulation causes eNOS de-palmitoylation (52) that induces translocation of the enzyme from the cell membrane to intracellular sites, uncoupling the protein from its activators, and thereby attenuating the formation and release of NO (Fig. 3). Following the decline in  $[Ca^{2+}]_i$  to basal levels, calveolin may once again interact with eNOS, leading to enzyme inhibition. Thus, the deactivation of the excitatory phase of this reaction is a highly specific sequence of events, highlighting its significance as well. Then, re-palmitoylation of eNOS facilitates rapid and efficient stabilization of the inactivated enzyme within the calveolar compartment and keeps it ready for a new cycle of stimulation by agonists (48,49) (Fig. 3). Such a mechanism may account for the spontaneous release of endothelial NO in a pulsatile mode that we observed *in vitro* from the median eminence of the hypothalamus, which is the common termination field of the adeno-hypophysiotropic systems. We

show that this hypothalamic area releases, without any drug addition or physical stress, one pulse of NO every 30 min and that the amplitude of the pulses varies significantly across the rat estrous cycle (Knauf, *et al.*, unpublished data). In summary, normal pulsatile blood flow induces the release of NO that is followed by another molecular mechanism accounting for its deactivation, allowing for the process to be initiated once more.

In this regard, it should also be noted, given the calcium dependency of cNOS, that normal calcium oscillations/pulsations may also contribute to the rhythmicity of NO release (53,54). Marhl and colleagues (53) develop a potential mechanism for calcium oscillations based on the balance of calcium stores in the endoplasmic reticulum (ER), mitochondria and cytosolic proteins. They surmise: 'The majority (approximately 80%) of calcium released from the ER is first very quickly sequestered by mitochondria. Afterwards, a much slower release of calcium from the mitochondria serves as the calcium supply for the intermediate calcium exchanges between the ER and the cytosolic proteins causing bursting calcium oscillations. Depending on the permeability of the ER channels and on the kinetic properties of calcium binding to the cytosolic proteins, different patterns of complex calcium oscillations appear'. This phenomenon coupled to the vascular pulsations may also explain the rhythmic burst of NO, which modulates its immediate environment.

**Nitric oxide microenvironment.** Prior exposure of human saphenous vein endothelial cells to NO decreased the adherence of immunocytes in response to several adhesion factors - interleukin (IL)-1 $\alpha$ , VCAM-1, IL-1 $\beta$ , IL-4, tumor necrosis factor (TNF- $\alpha$ ), or bacterial lipopolysaccharide (LPS) (55). It can also decrease the endothelial expression of E-selectin and secreted IL-6 and IL-8. These studies suggest that the effect of NO on cytokine synthesis may contribute, in part, to its anti-atherogenic and anti-inflammatory properties. In contrast, normal function may not allow these processes to develop. This study supports our concept that given our tonal/basal level of NO, the NO-coupled inhibitory role if lost, makes these cells more prone to excitation (18,23).

In part cNOS derived NO may be exerting this action via NF- $\kappa$ B, a transcription factor, which is essential for the activation of several inflammatory mediators, e.g., tumor necrosis factor (TNF)- $\alpha$ , interferon (IFN)- $\beta$ , IL-8, IL-1 $\beta$ , IL-2,

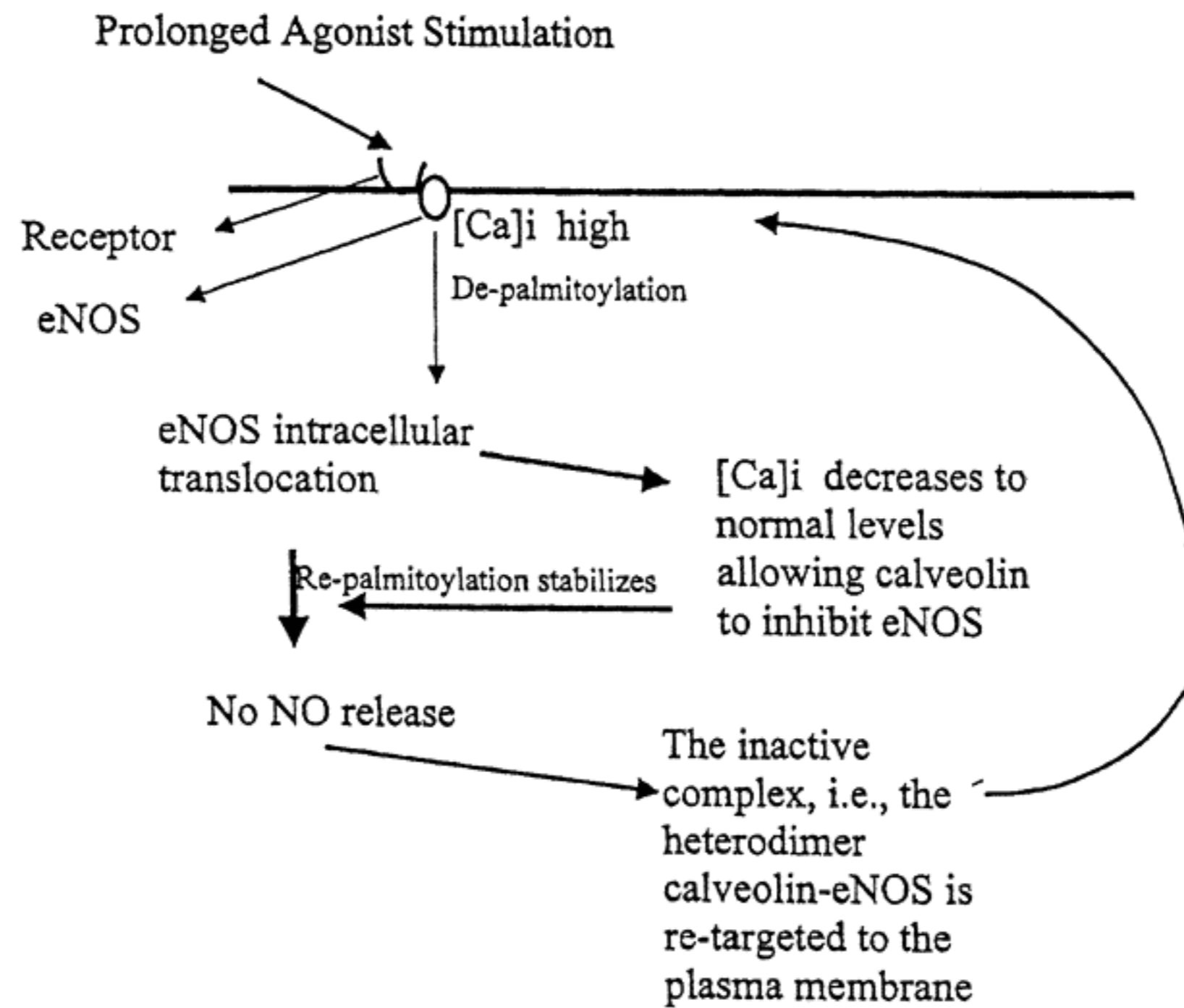


Figure 3 As a mechanism to counter the pressure stimulated NO release, prolonged agonist stimulation, in time, causes eNOS de-palmitoylation that induces translocation of eNOS from the cell membrane to intracellular sites, uncoupling the protein from its activators, and thereby attenuating the formation and release of NO. Following the decline in  $[Ca^{2+}]_i$  to basal levels, calveolin may once again interact with eNOS, leading to enzyme inhibition. Thus, the deactivation (relaxing) of the excitatory phase of this reaction is a highly specific sequence of events, highlighting its significance as well. Then, re-palmitoylation of eNOS facilitates rapid and efficient stabilization of the inactivated enzyme within the calveolar compartment and keeps it ready for a new cycle of stimulation by agonists.

and IL-6 (56). Upon NF- $\kappa$ B activation, in most types of cells, its inhibitor I $\kappa$ B- $\alpha$  is phosphorylated and proteolytically degraded (56). After activation, free NF- $\kappa$ B dimers are directly translocated into the nucleus where they bind to the promoter regions of target genes and induce transcription. A ~300-amino acid domain that is required for DNA binding and protein dimerization (56,57). Thus, NO, derived from eNOS, may tonically inhibit NF- $\kappa$ B under non-stimulated conditions (18,23,58,59). However, during advanced ageing coupled to a vascular factor that further reduces cerebral blood flow, this influence may be lost, allowing therefore, for greater sensitivity to activation.

Supporting this important hypothesis is that NO also stabilizes the NF- $\kappa$ B inhibitor, I $\kappa$ B- $\alpha$ , by preventing its degradation (60). The binding of NF- $\kappa$ B to the NF- $\kappa$ B binding site in the iNOS promoter plays a crucial role in the transcriptional regulation of the iNOS gene (61). Furthermore, NO donors can directly inhibit the DNA binding activity of NF- $\kappa$ B (62). In short, NO derived from eNOS, has the ability to down-regulate proinflammatory events via the inhibition of NF- $\kappa$ B activation of proinflammatory cytokines. We suggest that the morphine, IL-10, anandamide and estrogen down-regulation of cellular immune function occur by this pathway (18,37,58,59). Thus, specific stimuli that initiate eNOS NO release may prevent the appearance of deleterious/protective cytokines. One practical application of this information suggests that by liberating molecules that have the ability to stimulate eNOS and produce a quick burst of NO, the extent and the severity of a proinflammatory situation can be limited.

However, if the proinflammatory event was either initiated earlier or has become extremely strong, iNOS induction probably cannot be diminished. In this case, iNOS induction may represent a last attempt not only to overcome antigenic challenge but also to diminish a strong proinflammatory situation, which could result in biological damage and death.

**Nitric oxide and oxidative stress.** NO has the potential to interact with oxygen, metals and other free radicals (63). NO can form peroxynitrite (ONOO $\cdot$ ) and dinitrogen trioxide (N $_2$ O $_3$ ), following an interaction with the superoxide radical (O $_2^{\cdot-}$ ) and oxygen, respectively (64,65). In this regard, NO's direct effects are felt when its level is low and of short duration, thus occurring under physiological conditions (64). For example, NO interaction with the heme proteins represents the activation of soluble guanylyl cyclase (sGC) and/or cyclooxygenase (COX) (66-68). This last interaction is important in the regulation of a proinflammatory process (68). Additionally, at low NO concentrations it modulates the redox form of COX, converting the ferrous iron to the ferric active form, acting also as a scavenger of superoxide (64). NO also has the ability to inhibit lipoxygenase that can initiate tissue damage (67,69). It can also reversibly inhibit the heme moiety of cytochrome P-450, preventing the binding of oxygen to the catalytic sites (69,70). Interestingly, at low NO levels H $_2$ O $_2$  can be consumed to yield HNO $_2$  (71), counter-intuitively suggesting that H $_2$ O $_2$  might serve to control NO levels (64). In this regard, if NO were absent, H $_2$ O $_2$  may generate tissue

damage and energy metabolism may proceed impaired as occurs in AD (33).

Furthermore, as would be expected based on the above data, mitochondria represent a NO target since it is an inhibitor of cytochrome oxidase, a vital component of the electron transport process (71-78), suggesting a NO role in modulating oxygen utilization (73). The inhibition of cNOS-derived NO increases oxygen consumption in many animal species (79-83). This last fact is critical to our NO hypothesis. Furthermore, a NOS isoform, mtNOS, is present in mitochondria (72,84), suggesting an important modulatory function here as well.

Heme proteins (e.g., hemoglobin, cytochromes, etc.) reacting with  $H_2O_2$  results in ferryl cation ( $FE^{4+}=O$ ), a toxic substance (85). However, once in contact with NO, this compound is reduced ( $FE^{3+} + NO_2^-$ ) (64), demonstrating a NO anti-oxidant action. NO also has the potential to diminish the formation of OH $\cdot$ , demonstrating once more an anti-oxidant action (86). This scavenging property gives NO a major intracellular and extracellular action against oxidative stress (64,87-93). Here again we note that in the absence of NO these reactive chemical species may cause tissue damage associated with various neurological pathology, including energy metabolism alteration at the level of the mitochondria.

### 3. Exercise

In our model we surmise that mild exercise is highly beneficial because, based on the above discussion, it promotes cNOS derived NO release in a pulsatile rhythmic pattern. This phenomenon stabilizes the vascular endothelium, i.e., not permitting gap formation between the cells (43) (Fig. 4). It also serves to keep the endothelial cell in a mild state of inhibition, reducing the potential influences of environmental noise. NO so produced can modulate the microenvironment in regard to controlling the balance of free radicals. This also serves to stabilize the immune system that strongly interacts with the vascular endothelium during a proinflammatory event. That is, if the endothelium is down-regulated, immune cells will not be able to adhere in large numbers thus blocking or diminishing immunocyte trafficking.

As noted at the beginning of the review, severe or enhanced exercise tends to resemble a proinflammatory response in that sustained high vascular pressure may or will not promote cNOS derived NO release, allowing, in time, for the activation of proinflammatory cytokines, cellular adhesion, cellular conformational changes, i.e., endothelial, that if allowed to continue may initiate immune and/or vascular pathology (Fig. 5). In this regard, for example, NO cannot inhibit NF- $\kappa$ B activation. Indeed, enhanced free radical generation may also serve to scavenge NO, further enhancing a proinflammatory state. We surmise, that this insult can only occur for so many times before the compensatory processes that serve to correct it can accomplish this end. Thus, this form of exercise can become progressively debilitating, showing its worst characteristics during old age (Fig. 5). We speculate that this form of exercise is abnormal, not only based on the proinflammatory potential, but because other animals normally exert themselves in spurts of activity not sustained endeavors designed to enhance performance for performance's sake.

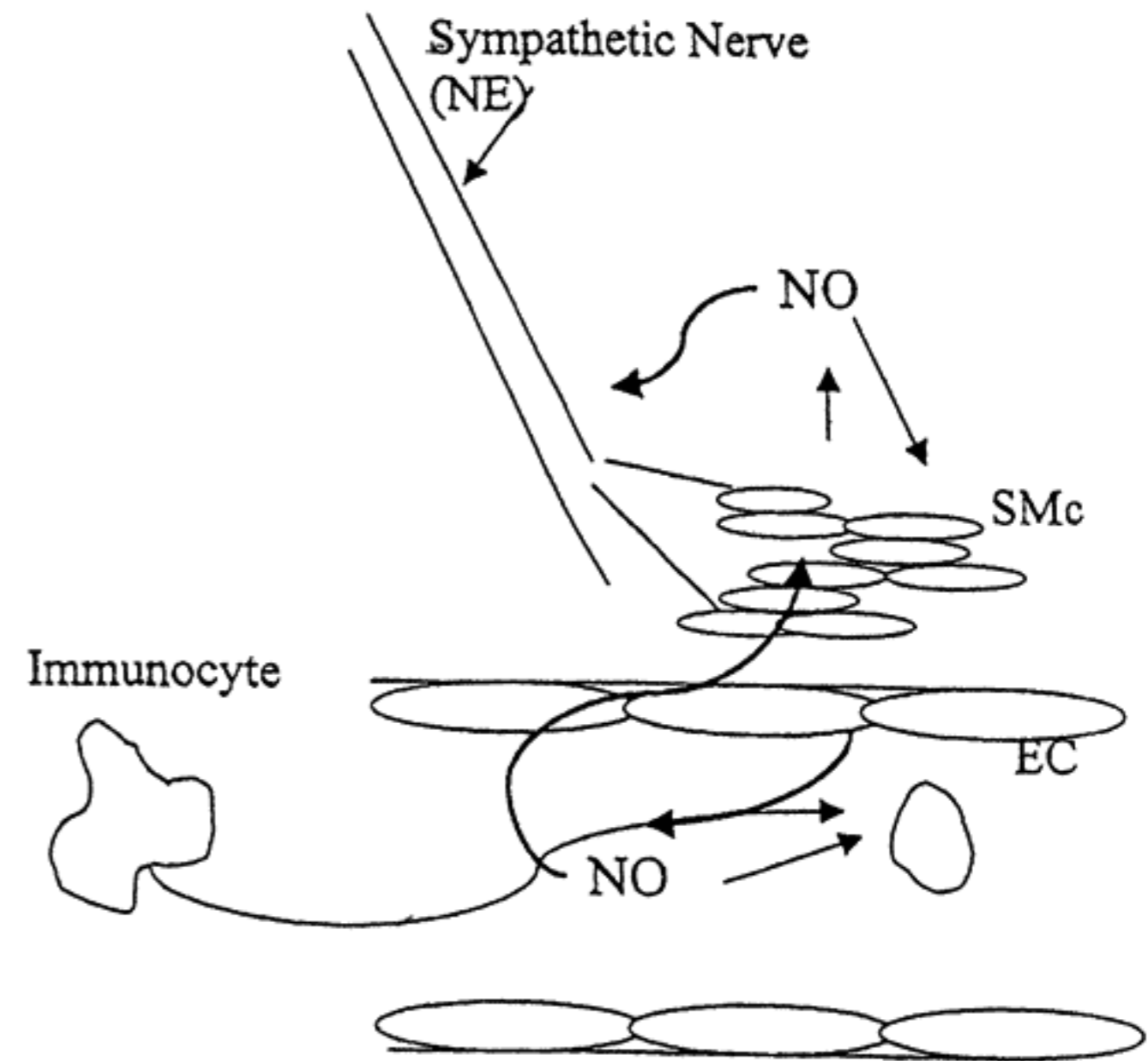


Figure 4. Noradrenergic (NE) neurons in peripheral vascular arterial tissues innervate the smooth muscle cells (SMC) Nitric oxide (NO) via cNOS derived NO release and agonist stimulated release can down-regulate the endothelium, making it a less desirable substrate of immunocyte adherence. Such NO from immunocytes also can down-regulate the endothelium as well as the immunocytes themselves, making them round and inhibiting adhesion molecule expression. NO can inhibit NE release in peripheral vascular tissues as well as stimulate SMC degranulation of F-actin (121). Thus, NO that can also scavenge free radicals also may act to normally dampen NE vascular processes simultaneously protecting them from free radical damage. This is what appears to be occurring on a constitutive level to maintain normal resting conditions. We surmise normal exercise induced perturbations alter these processes, i.e., overcoming the NO down-regulating action by a rapid neuronal infusion of released NE (Fig. 5).

Given this, the potential for mild exercise via cNOS derived NO to maintain a general down-regulated physiological state involving the immune and vascular system, it would not be surprising to find that light or mild exercise, promoting rhythmic NO release, would be found beneficial in particular biomedical disorders (Fig. 4). Conversely, biomedical conditions exhibiting a diminished capacity in this overall phenomenon would be at risk for progressive debilitations, i.e., Alzheimer's disease (43) (Fig. 5). Furthermore, as noted below, we surmise that instituting a program of mild cyclic exercise, mimicking natural activity patterns, may promote health as well as restore health (this process) to a working condition where it provides a health benefit to the individual. We are specifically targeting the cNOS derived NO release mechanism. In this regard, we also surmise that if a particular system is compromised organically to such an extent, this exercise protocol may not work. Thus, as with all therapies, it offers great promise to a subset of the potential population. Here, we now examine a few disorders to determine if the mild therapeutic exercise approach can be beneficial.

### 4. Pathology

*Aging.* Resistance to blood flow is in the microcirculation (94) and normal aging is associated with increased vascular

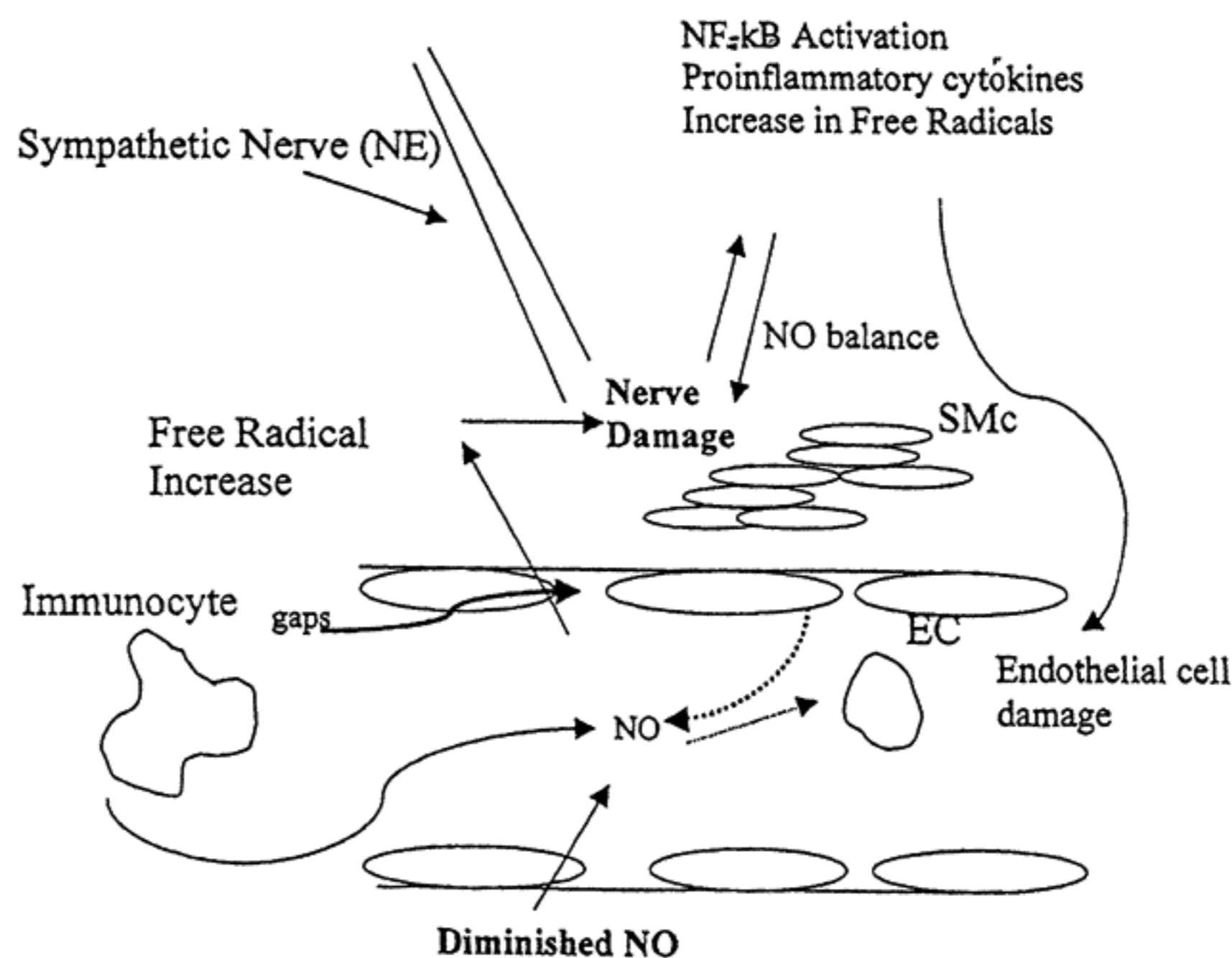


Figure 5. Illustration of what may happen under severe exercise and/or pathological conditions. As a result of diminished nitric oxide (NO) endothelial cells change shape revealing gaps between endothelial cells and a thickening of the basement membrane, altering the flow of nutrients and thus affecting energy metabolism (see text and Fig. 2). In the microenvironment of low basal NO (dotted arrows) free radicals may accumulate, initiating tissue damage, especially involving neurotransmitter nerve peripheral varicosities, i.e., norepinephrine. This condition may be made worse due to the diminished supply of NO since NF- $\kappa$ B processes are no longer inhibited. This would tend to lead to proinflammatory expression and an even greater increase in microenvironmental free radicals. Thus, as this process begins, its negative sphere of influence would grow with time and become progressively worse. Indeed the general, or progressive lack of activity associated with Alzheimer's and Parkinson's disease may be the underlying event responsible for the development of this scenario. Given this, mild exercise may reverse these negative changes, allowing various tissues to re-establish their NO autoregulatory loops (121).

resistance (95,96). Examination of autopsied brains revealed that thickening of cerebral arteries at age 5-25 is possible but rather uncommon, while such vasculopathy is found in 80% of those aged 80 (97). Taken together, these histological changes associated with aging may be the result of diminished NO production that would then affect energy metabolism, cell shape etc. as indicated in the earlier discussion.

**Alzheimer's disease.** In a recent review concerned with Alzheimer's disease we have discussed a vascular origin hypothesis (33). It centers on a 'critically-attained threshold of cerebral hypoperfusion' (CATCH) syndrome. Briefly, Alzheimer's disease begins as aging individuals with a pre-morbid condition in the form of a vascular risk factor develop, usually over a considerable time period, a CATCH that initiates regional brain capillary degeneration. Clinical evidence suggests that three interactive factors are fundamental in the development of CATCH: i) duration of hypoperfusion; ii) severity of hypoperfusion; iii) age of subject (33). The collective findings suggest that prolonged brain hypoperfusion brought on by CATCH, directly produces an alteration of basal NO levels, possibly as a result of the loss of pulsatile flow, that over time, due to the compensatory momentary stimulation of eNOS by signal molecules, results in a gradual loss of basal NO microenvironmental regulation. This may anatomically manifest itself initially as an alteration of endothelial cell shape and a compensatory thickening of the basement membrane to compensate for the change in vascular permeability, resulting from endothelial 'gaps' (33).

A considerable number of pathologic studies have shown that brain capillaries in Alzheimer's disease develop basement membrane thickening, pericyte degeneration, endothelial cell shape changes, and luminal buckling (98,99). This microvascular pathology is present in virtually all AD brains examined (100). According to fluid dynamic laws, these capillaries will transform normal laminar flow into disturbed flow at regions where structural changes in the microvessel's cylindrical lumen occur (98,99). Progressive and modest changes in the microvasculature can then promote a decrease in glucose and oxygen delivery to neuro-glial cells (33) (Fig. 4). Additionally, the diminished delivery and transport of these energy substrates would contribute to regional capillary deterioration, partially by generating free radicals that may scavenge NO, further diminishing its ability to regulate the cellular microenvironment. The outcome of CATCH, therefore, would promote regional endothelial cell dysfunction and impair eNOS homeostasis which in time initiates the energy metabolic disturbances that lead to Alzheimer's disease and progressive neurodegeneration. Thus, we hypothesize that mild exercise may lead to an improvement of this condition, especially if diagnosed early on.

**Parkinson's disease (PD).** In recent times there has been an increase in the number of scientific reports documenting the positive effects of exercise on PD patients (101-106). In this regard, while exercise improved PD performance, if normal activity was resumed the benefit was lost (102). How can we explain these observations? In a recent review it is noted

that cardiovascular dysfunction is relatively common in Parkinsonian patients (107). This has been associated with an impairment of autonomic function (108). Also noted among Parkinsonian patients is a disorder of multiple system atrophy (MSA) and orthostatic (postural) hypotension (109). In this regard orthostatic hypotension is critically dependent upon an adequate perfusion pressure, e.g., a drop in systolic blood pressure of approximately 20 mm Hg, or in diastolic of 10 mm Hg, on either standing or head-up tilt to at least 60° (110). In other Parkinsonian patients autonomic failure (AF) may be evident that may also lead to vascular dysfunction (111-113).

In normal individuals vasodilation occurs in exercising skeletal muscle even during a modest degree of exercise. In PD, the management of exercise-induced hypotension currently is unsatisfactory (114). In PD sustained hypertension may result. In this regard, we surmise normal blood pulsing is limited, thus over time there would be a decrease in eNOS derived NO, a condition that may further exacerbate PD associated neural damage, i.e., increased  $\alpha$ -adrenergic pressor supersensitivity (115) (Fig. 5). This may originate from a lack of NO balancing the NE pressor effect or sympathetic vascular degeneration (116). As noted by Mathias: 'It is unclear why cardiac sympathetic denervation should occur at a relatively early stage in PD (107)' unless as we propose, it is an alteration of NO regulation of NE release (116,117) (Fig. 5). Thus, there may be an intrinsic propensity to the development of arrhythmias, especially in PD, because of sympathetic cardiac alteration associated with NO.

As also noted by Mathias (107), cerebral hypoperfusion can produce a variety of symptoms that can be found in other conditions like AD above and transient ischemic attacks in carotid artery stenosis (118,119). In MSA, basal levels of skeletal muscle blood flow are higher than in normal subjects (120). There is a greater rise in blood flow in exercising muscle in MSA than in normal subjects, and the levels remain elevated for longer after cessation of exercise, presumably because of impairment of corrective autonomic neural responses. Indeed, this can also be understood from a vascular pulsation point of view. Here, due to poor perfusion eNOS-associated NO release is impaired and in time negatively alters sympathetic vascular NE function, by allowing free radicals to damage the vascular neural fibers. Thus, when called upon to dilate, the vascular pulsations are working almost exclusively on endothelial cells eNOS, which when liberated initiates vasodilation, however, by not being balanced by NE, the dilation period is extended (Fig. 5). This would account for the apparent sympathetic denervation with pressor supersensitivity to small amounts of endogenous NE, which emerges with PD (107).

## 5. Conclusion

Given this, how can we account for the beneficial effects of exercise? We speculate that cyclic exercise, initiating blood pulsatile flow restores the endothelial cells to a near normal homeostasis by re-establishing the eNOS derived NO regulated environment. In doing this, the restoration of NO balance may serve to decrease the presence of free radicals, allowing the vascular endothelium to return to a state of inhibition while simultaneously protecting sympathetic nerve

endings. Indeed, this process may be vital in sustaining the life of the dopaminergic neurons in the substantia nigra. However, given the inherent weakness in the condition that initiated vascular associated PD, we surmise that cyclic exercise can only offer a beneficial effect as long as it is practiced. This hypothesis also assumes that cyclic exercise will not work in the last stages of PD or AD because the vascular endothelium may be organically compromised to a point of irreversible damage. If we are correct, it becomes important for these individuals to enter into mild cyclic exercise programs early on. Only with early intervention can the progressive debilitating processes be reduced, delayed and possibly inhibited. We also surmise that stressful exercise programs will not provide the therapeutic benefit because they do not allow for pulsations, since they tend to maintain a steady hypertensive state, not allowing eNOS derived NO to be released, generating a potentially dangerous proinflammatory state. This hypothesis also leads one to conclude that mild exercise may prove to offer physical help while providing psychological relief to these individuals. It also offers an affordable alternative to other forms of therapy.

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