Nitric Oxide Stimulating Dietary Supplements:
Where is the evidence?

Nitric Oxide Supplements

ABSTRACT

Dietary supplements purported to increase nitric oxide production have gained enormous popularity within the athletic (e.g., bodybuilding) community in recent years. Although anecdotal reports suggest a potential benefit from using such products, the scientific evidence is sparse. To the knowledge of this author, only one ingredient consumed in oral form by human subjects has been reported within the scientific literature to promote an increase in blood nitric oxide. This ingredient is a unique form of L-carnitine called glycine propionyl-L-carnitine (GPLC). This article discusses the scientific evidence for nutritional supplements to increase nitric oxide production, as well as the physiological rationale as to why nitric oxide may be important related to athletic performance. Directions for future scientific work in this area of sport supplementation are presented.

INTRODUCTION: WHAT IS NITRIC OXIDE?

Nitric oxide ($NO^{\cdot}$), first referred to as endothelium derived relaxing factor (Furchgott and Zawadzki, 1980), is biosynthesized from the amino acid L-arginine, oxygen, and a variety of cofactors, by nitric oxide synthase enzymes (Collier and Vallance, 1991). Nitric oxide is a gaseous chemical compound that acts as an important signaling molecule within the human body, known to facilitate a variety of critical functions as previously described in detail (for recent reviews please see Bian et al., 2008; Thomas et al., 2008). In this regard, high concentrations of $NO^{\cdot}$ favor cell cycle arrest and apoptosis, while brief production at low (nanomolar) concentrations favor a wide array of beneficial physiological functions including enhanced blood flow and immune defense, decreased platelet and leukocyte adhesion, decreased smooth muscle cell proliferation, regulation of neurotransmission and muscle atrophy/hypertrophy, and the stimulation of satellite cells (Anderson, 2000; Bian et al., 2008; Salanova et al., 2008; Thomas et al., 2008). These effects appear mediated via both a cyclic guanosine monophosphate (cGMP) dependent and independent signaling cascade (Bian et al., 2008).

In relation to sport supplementation, $NO^{\cdot}$ is of great interest primarily related to the ability of this molecule to facilitate vasodilatation, by acting on vascular smooth muscle cells (Bian et al., 2008). This major discovery, initially by Furchgott and Zawadzki (1980), has led countless other scientists from around the world to devote their research agendas to work related to $NO^{\cdot}$. In fact, $NO^{\cdot}$ was recognized as "molecule of the year" by *Science* magazine in 1992, and the Nobel Prize in Physiology or Medicine was awarded in 1998 to Robert Furchgott, Louis Ignarro, and Ferid Murad (with the unfortunate omission of Salvador Moncada) for their work related to $NO^{\cdot}$ as a signaling molecule within the cardiovascular system. Over the past 5 years in particular, $NO^{\cdot}$ has received significant attention from the athletic community (primarily bodybuilders), fueled largely by the aggressive marketing campaigns of sport supplement companies who manufacture products touted to "stimulate nitric oxide production". In this regard, the primary desired effect is the potential increase in blood flow to active skeletal muscle, mediated by the claimed increase in $NO^{\cdot}$. It is hypothesized that this proposed increase in blood flow would then lead to an increase in oxygen and nutrient delivery (e.g., amino acids and glucose) to skeletal muscle in order to aid exercise performance and to help facilitate recovery.

EXERCISE AND NITRIC OXIDE

Before considering the impact of a dietary supplement on $NO^{\cdot}$ production, it is important to understand the role of exercise itself in facilitating increased $NO^{\cdot}$. This is most often determined by the combined measurement of nitrate (NO_3^-) and nitrite (NO_2^-) in blood or urine, which are considered stable products of the rapidly degraded $NO^{\cdot}$ (half life equal to 3-4 seconds).

Previous reports indicate that acute exercise results in increased blood $NO^{\cdot}$ (Bode-Boger et al., 1994a; Clarkson et al., 1999; Rognmo et al., 2008), a finding evident for both dynamic (Hickner et al., 1997) and isometric (Gilligan et al., 1994) exercise. Moreover, studies involving chronic exercise training performed 3-4 days per week have also noted an increase in resting levels of $NO^{\cdot}$ (Edwards et al., 2004; Poveda et al., 1997; Tordi et al., 2006). These findings may help to explain some of the positive health and performance outcomes apparent in exercise trained individuals compared to their sedentary counterparts. Hence, what may be perhaps the most important initial recommendation is the performance of regular, structured exercise by all individuals interested in reaping the potential benefits of increased circulating $NO^{\cdot}$.

While $NO^{\cdot}$ is indeed thought to play a role in enhanced blood flow during an acute bout of exercise (again, the chief marketing point of most sport supplement companies selling such products), other mechanisms are indeed involved (e.g., flow mediated dilation, muscle contraction-induced distortion of resistance vessels) as previously described (Joyner and Wilkins, 2007). In fact, these other mechanisms are believed to be primarily responsible for allowing for optimal blood flow redistribution and hyperemia with acute exercise, with $NO^{\cdot}$ playing only a minor role (Tschakovsky and Joyner, 2008). Therefore, even if such nutritional supplements did result in a significant and measureable increase in $NO^{\cdot}$, the question becomes, would this have any physiological relevance in the context of an acute exercise bout? Clearly, no data presently exist to address this question.

PHARMACEUTICALS, DIETARY SUPPLEMENTS AND NITRIC OXIDE

Aside from exercise, pharmaceutical agents have been used with success to either induce $NO^{\cdot}$ biosynthesis or to enhance/maintain the actions of $NO^{\cdot}$, with the primary end goal of promoting vasodilatation (Burgaud et al., 2002). These include nitrates (transdermal long acting, sublingual rapid acting), propionyl-L-carnitine (intravenous), and L-arginine (intravenous), as well as agents used for the treatment of erectile dysfunction (e.g., Viagra®, Cialis®). In most cases, the above drugs are used to treat ailments involving impairments in blood flow including various forms of cardiovascular disease, such as angina arising from myocardial ischemia, as well as peripheral arterial disease.

In some studies, treatment with high dose L-arginine, the $NO^{\cdot}$ precursor amino acid, has been associated with enhanced vasodilatation (Bode-Boger et al., 1994b; Giugliano et al., 1997). However, it should be noted that the route of administration in such studies has been intravenous injection and not oral intake. In fact, a review of studies involving oral intake of L-arginine at dosages ranging from 10-20 grams indicates no benefit of this amino acid with regards to either increased circulating $NO^{\cdot}$ or enhanced blood flow (Adams et al., 1995; Chin-Dusting et al., 1996; Robinson et al., 2003). In addition, an oral dosage of only 10 grams per day has been noted to have an unpleasant taste and in some cases result in gastric distress (Robinson et al., 2003). It is evident that sport supplement companies are simply basing their “research” related to their products on investigations involving intravenous injection of L-arginine. Obviously, the route of administration is important in this regard. For example, work involving direct comparisons between intravenous and oral intake of L-arginine indicates no effect of oral L-arginine intake on vasodilatation, partly due the fact that oral L-arginine bioavailability is only ~68% and varies across subjects (Bode-Boger et al., 1998). Moreover, while the dosage of L-arginine used in intravenous studies has often ranged from 6-30 grams given in a bolus dose, most of the dietary

supplements sold on the market today include only 3 grams of arginine per serving. At this low dosage, it is highly unlikely that oral intake of such supplements will have any impact on $NO^{\cdot}$ or on the desired changes fostered by the proposed increase in this molecule.

Equally important, although L-arginine is the precursor to $NO^{\cdot}$ biosynthesis, it has been suggested that this amino acid is not the rate limiting component (Kurz and Harrison, 1997). Rather, nitric oxide synthase enzymes may be most important to $NO^{\cdot}$ biosynthesis. Therefore, adding excess L-arginine may do little to promote increased $NO^{\cdot}$ production, unless individuals have enzymatic insufficiency. In fact, a recent report indicates that 3 grams per day of L-arginine results in no increase in $NO^{\cdot}$ availability and actually reduces exercise time to fatigue (Wilson et al., 2007). Any anecdotal effect that individuals claim to experience when using many of the marketed products may be more dependent on the sugar contained within the product, rather than the L-arginine. This is because sugar intake results in an insulin spike, and insulin itself has been shown to yield a vasodilating effect (Giugliano et al., 1997; Steinberg et al., 1994). In fact, intravenous L-arginine itself at a dosage of 30 grams over 30 minutes has been shown to increase insulin release (Giugliano et al. 1997) and promote vasodilatation. It is possible that some "cocktail" products which contain several ingredients aside from sugar and L-arginine may promote an effect on $NO^{\cdot}$ and subsequent vasodilatation. However, only well designed, controlled scientific studies will determine whether or not such products can substantiate the radical claims currently being made for such products.

Despite the lack of scientific data, it is evident that dietary supplements marketed to increase $NO^{\cdot}$ production are rampant within the supplement industry. In fact, a quick scan of many of the popular bodybuilding magazines indicates that in any given month there are 30 or more entire pages of advertisements devoted solely to this class of dietary supplement. Indeed, this is an area of interest with the athletic community.

GLYCINE PROPIONYL-L-CARNITINE AND NITRIC OXIDE

One novel ingredient that has emerged as a potential candidate to result in a measureable increase in $NO^{\cdot}$ production is glycine propionyl-L-carnitine (GPLC). GPLC is a molecular bonded form of propionyl-L-carnitine and the amino acid glycine. We have recently reported in previously sedentary men and women (Bloomer et al., in press) and in resistance trained men (Bloomer et al., 2007) that oral intake of GPLC at a dosage of 4.5 grams per day results in increased plasma $NO^{\cdot}$, as measured by $NO_3^- + NO_3^-$. Our findings agree with other recent work using PLC exclusively (Lofreddo et al., 2007) which demonstrated an increase in blood $NO^{\cdot}$ in response to 6 grams per day of PLC given via intravenous infusion.

The mechanisms of action for this effect appear mediated by a decrease in NADPH oxidase activation (Pignatelli et al., 2003), which subsequently leads to superoxide radical generation (Zalba et al., 2001) and decreased $NO^{\cdot}$ bioavailability. It has also been reported that PLC augments endothelial nitric oxide synthase (eNOS) (de Sotomayor et al., 2007), leading to increased $NO^{\cdot}$ production. Additional work to investigate the mechanisms associated with the increase in plasma $NO^{\cdot}$ with GPLC treatment is needed.

CONCLUSION

With the exception of two published studies reporting an increase in plasma $NO^{\cdot}$ with oral intake of GPLC (Bloomer et al., 2007; in press), there exist no published reports to indicate that the dietary supplements currently being marketed as "nitric oxide stimulators" have proven efficacy. Several interesting research questions remain to be answered with regard to this class of dietary supplement. This includes GPLC, for which enhanced performance and recovery related to exercise has yet to be noted. For example, can such products 1) Stimulate an increase in $NO^{\cdot}$ production? 2) Stimulate an increase in blood flow? 3) Stimulate an increase in oxygen and nutrient delivery to target tissue? 4) Enhance

aerobic and anaerobic exercise performance? 5) Enhance exercise recovery? and/or 6) Enhance muscle mass? Only continued funding by committed supplement companies will provide the opportunity for these and other questions to be addressed. Without such work, and subsequent evidence showing measureable improvements in these variables, this field will remain much more hype than effect. Current marketing campaigns by many of the top sport supplement companies are presented as though these questions have already been answered in the affirmative. Clearly, this is not the case.

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