

Effects of an H₂-infused, Nitric Oxide-Producing Functional Beverage on Exercise and Cognitive Performance

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Background. Exercise performance and recovery are impaired by excessive levels of oxidative stress and inflammation. However, both reactive oxygen species (ROS) and inflammation improve exercise performance including mitochondrial ATP production and force of muscle contraction. They also are essential mediators in providing the benefits and training adaptations that occur from exercise. Nitric oxide (NO[•]) is a gaseous radical that increases blood flow via dilation of the blood vessels and also improves mitochondrial function. Therefore, NO[•] improves exercise performance and capacity, but only when produced at the right times and in the right locations. Excessive levels of NO[•] contribute to nitrosative stress due to the spontaneous reaction with superoxide to form toxic peroxynitrite. This decreases the ½ life of nitric oxide resulting in less NO[•] benefits and in cellular damage leading to impaired exercise performance. In contrast to conventional antioxidants and anti-inflammatories, molecular hydrogen (H₂) has been demonstrated to exert selective antioxidant and anti-inflammatory effects by decreasing only excessive inflammation and reducing toxic oxidants without disturbing important signaling ROS, like NO[•]. Moreover, H₂ can regulate NO[•] production, increase its circulating ½ life and beneficial NO[•] cycling, potentiate the bioactivity of NO[•], and act as a NO[•] mimetic by increasing cGMP levels. At the same time H₂ can prevent peroxynitrite formation and reduce the harm from NO[•] metabolism, such as lower nitrotyrosine levels. **Methods.** The effects of an H₂-infused, nitric oxide-producing beverage (Hydro Shot) on nitric oxide production, blood flow, aerobic and anaerobic exercise, and cognitive function were assessed. **Results.** Ingestion of the functional beverage significantly increased production of NO[•] and a concomitant increase in blood flow. It also improved aerobic performance as measured by VO₂, and anaerobic performance as indicated by delayed muscle fatigue, and increased peak torque during maximal isokinetic leg extensions. Additionally, the H₂/NO[•] combination significantly improved indices of cognitive function including, focus, speed, plasticity, etc. **Conclusion.** The molecular crosstalk between H₂ and NO[•] coupled with these preliminary results indicate that Hydro Shot is uniquely qualified for sports performance and exercise medicine and warrants additional clinical and mechanistic research.

Citation

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Introduction

Exercise has many beneficial effects, but when done in excess or not regularly, it increases harmful inflammatory and oxidative stress. Excess inflammation and oxidative stress impair exercise performance, reduce mitochondrial activity, decrease force of muscle contraction, increase rate of fatigue, and lead to slowed recovery, overtraining syndrome, and injuries. Unfortunately, supplementation with conventional antioxidants and/or anti-inflammatories have not been shown to protect against the harmful effects of oxidative stress and inflammation. Worse, such supplementation may actually negate the benefits of exercise training. This is because, although oxidative stress and inflammation are pathological in excessive and chronic levels, lower levels are critical for growth, repair, and recovery. Conventional antioxidants/anti-inflammatories are neither selective nor very effective at combating oxidative stress and inflammation as they lack cellular bioavailability, selectivity, kinetic diffusivity, and homeostatic modulatory activity. However, molecular hydrogen, especially in combination with nitric oxide, has the needed properties to be an effective modality.

Hydro Shot is a zero-calorie, functional beverage containing nitric-oxide-stimulating citrulline that has been infused with molecular hydrogen. The concentration of H₂ is above 2 mg/L as determined via gas chromatography (H₂ Analytics, Las Vegas, USA; SRI 8610C; California USA). All ingredients have a high safety profile and are designated as GRAS (Generally Recognized as Safe) by the USFDA. The novel combination of nitric oxide with molecular hydrogen makes it an ideal drink for sports performance. Consumers of such product have reported a wide range of both health benefits and improvements in athletic performance.

Molecular hydrogen (H₂), and nitric oxide (NO[•]) separately have been the subject of numerous research studies to determine their antioxidant and anti-inflammatory properties and general health benefits, but their combined effects have not been widely explored. Research has shown that molecular hydrogen can be an effective therapy for oxidative stress, a major contributor to many diseases, aging, and impaired exercise performance. Nobel Prize-winning research has shown that nitric oxide is a signaling molecule of key importance for the cardiovascular systems and it helps regulate blood pressure. Testing has shown that when the H₂/NO[•] combination is consumed, a combination of independent and synergistic actions of H₂ and NO[•] take place, resulting in extended energy, improved performance, and therapeutic benefits to certain health conditions.

The scientific rationale for these ergogenic effects is briefly discussed based on the peer-reviewed scientific literature. We also provide some preliminary human data on the aforementioned functional beverage that indicates impressive athletic and cognitive improvements.

Molecular Hydrogen

Molecular Hydrogen (H₂) which is naturally produced by intestinal bacteria, has recently emerged as a therapeutic medical gas [1]. It is administered clinically primarily either via inhalation or dissolved in water to create hydrogen-rich water (HRW) [1]. In Japan, molecular hydrogen has been approved as an advanced medicine for the treatment of post-cardiac arrest syndrome, for which a 360 patient, multi-center, clinical study is being conducted [2]. This was preceded by a 2007-landmark publication in *Nature Medicine* [3], which demonstrated that H₂ significantly suppressed brain damage caused by a stroke in rats. Although the research on H₂ is still in its infancy, the nearly 2,000 scientific publications including (≈100 human clinical studies), have continued demonstrating favorable biological effects. Human clinical studies have demonstrated enhanced antioxidant status [4,5], reduced inflammation [5], improved cholesterol levels [5,6], decreased sympathetic nerve activation [7], and improved athletic performance [8]. The exact molecular mechanisms responsible for these biological effects are still being investigated, but it is clear that H₂ has antioxidant, anti-inflammatory, and anti-apoptotic protective effects. This is done via by modulating signal transduction, influencing gene expression, and modulating protein-phosphorylation cascades [1].

Bioavailability

This tasteless, odorless, and flammable gas has the highest rate of diffusion, which makes it attractive for human biology. In order for drugs, supplements, or any nutraceutical/pharmaceutical to have any biological effect, they must first be absorbed by the body and permeate into the cells. Bioavailability depends primarily on three properties i) size, with the smaller the molecule the more bioavailable, ii) charge, with neutral molecules being more bioavailable, iii) polarity, with non-polar molecules being more bioavailable. Accordingly, most drugs have limited absorption and require transport mechanisms in order to enter the cells, which limits both the concentration and the rate that they penetrate into the cell. In contrast, H₂ is the smallest molecule in the universe, and is a neutral and non-polar molecule, which affords it the highest bioavailability of essentially any other molecule. H₂ also has the highest rate of diffusion, and upon ingestion, it reaches systemic circulation within seconds, and penetrates the organs and cells within 2-10 minutes. The high cellular bioavailability and beneficial biological effects of molecular hydrogen uniquely qualify it as an optimal ergogenic molecule for exercise medicine and sports performance.

Selectivity and Exercise Benefits

In contrast to conventional antioxidants, H₂ not only has higher bioavailability but has unique selectivity against reactive oxygen species and inflammation. Just as not all forms of cholesterol are bad (HDL good; LDL bad), neither are all reactive oxygen species (ROS) and inflammation. As mentioned, many forms of ROS and inflammation are what mediate the benefits of exercise. H₂ is selective and only reduces the toxic and harmful ROS (e.g., hydroxyl radicals, [•]OH, peroxyne (ONOO[•])), but does not, indeed cannot, neutralize beneficial signaling ROS including hydrogen peroxide (H₂O₂), superoxide (O₂⁻), nitric oxide (NO[•]), etc. Similarly, in contrast to conventional anti-inflammatories, which can also negate exercise training benefits, H₂ is a mild modulator of inflammation. Moreover, it modulates multiple inflammatory pathways (e.g., NF-κB, cFOS, NFAT, miRNAs, etc.) and not just on one protein (e.g., NSAIDs inhibit cyclooxygenase), and has many of these effects at the level of gene expression. We are not what our genes are, but how our genes are expressed. Therefore, H₂ does not have the same inherent risks as other antioxidants/anti-inflammatories. Accordingly, research indicates that H₂ can protect against excessive exercise training while not hampering the benefits of exercise training.

Congruously, human studies on exercise performance with H₂ have demonstrated a number of favorable responses such as decreased lactate production, delayed fatigue, and even as a treatment for soft tissue injuries [9] Recently published in this journal is a case report indicating that supersaturated HRW hydrotherapy improved recovery of an acute musculoskeletal injury [10]. Many studies have demonstrated important and diverse benefits of H₂ supplementation, which are briefly summarized in Table 1. (see [8] for more details).

Exercise Benefits	
Improved endurance via VO ₂ ,	[11-13]
reduced psychometric fatigue, increased exercise time	[11]
Decreased exercising heart rate during	[14,15]
Normal homeostatic inflammatory response to exercise	[16]
Normal homeostatic ROS/antioxidant response to exercise	[17]
Improved natural antioxidant response to exercise	[18]
Reduced markers of exercise-induced DNA damage	[19,20]
Improved microbiome and antioxidant and anti-inflammatory markers	[21]
Reduced lactate levels	[13,15,17,22]
Maintained peak-power output during repetitive sprints to exhaustion	[23]
Reduced pain and swelling with a faster recovery in range of motion	[9,10]
Reduced delayed onset muscle soreness	[24]

Table 1. Brief summary of clinical studies on exercise benefits of H₂ administration

Many animal studies have shown similar benefits and, upon tissue and cellular analysis, additional benefits that directly benefit athletic performance have been reported. A summary of these benefits and properties includes increased levels of PGC-1 α [25], a marker of mitochondrial biogenesis, enhanced endogenous antioxidant and detoxification enzymes [26], and induction of sirtuin-3 [27], which largely mediates many benefits of exercise training. Additionally, H₂ can: a) rapidly reach subcellular compartments via passive diffusion and protect DNA, RNA, proteins, cell membranes, and mitochondria from damage [28]; b) selectively decrease only the most cytotoxic ROS without eliminating the beneficial-signaling ROS [3]; c) maintain redox homeostasis by decreasing the oxidant load via cell modulation (e.g. downregulation of the NADPH oxidase system) [29]; d) decrease excessive levels of pro-inflammatory mediators (e.g. cytokines, COX2, NFAT, etc.); e) maintain mitochondrial membrane potential [30]; and f) and increase ATP production [31], all of which can provide an ergogenic effect for athletes.

Nitric Oxide

Nitric oxide (NO \cdot) is a potent signaling molecule that influences an array of physiological responses. It is present in most living creatures and is made by many different types of cells. When produced by the endothelium (innermost layer of the arteries), it rapidly spreads through the cell membranes to the underlying muscle cells. Their contraction is turned off by the nitric oxide, resulting in dilation of the arteries. Nitric oxide is primarily produced via nitric oxide synthase (NOS), of which there are three isoforms, namely, inducible NOS (iNOS), neuronal NOS (nNOS), and endothelial NOS (eNOS) [32]. The amino acid arginine is metabolized by this enzyme to produce NO \cdot and citrulline. NO \cdot levels gradually decrease with age; up to 75% in 70-80-year-olds compared to healthy 20-year-olds [33]. Even under normal conditions, arginine is quickly metabolized; however, supplementation with citrulline is more effective at increasing plasma arginine and NO \cdot levels compared to supplementing with arginine [34]. Low levels of citrulline may also lead to NOS uncoupling, which induces further oxidative and cellular damage [35].

Nitric oxide and molecular hydrogen combination

The combination of nitric oxide and molecular hydrogen in a sports beverage may work synergistically to enhance exercise performance. There is significant crosstalk between these two gaseous-signaling molecules [36]. There are several ways that molecular hydrogen may have synergistic and potentiating effects with NO \cdot . For example, unlike conventional antioxidants H₂ does not react with or neutralize the NO \cdot radical. It may also enhance nitric oxide's biological activity and conversion of citrulline to nitric oxide. However, excess levels of nitric oxide, especially when produced from the iNOS, can lead to oxidative and nitrosative stress, which directly impairs exercise performance. H₂ regulates NO \cdot production and activity, thus preserving and even potentiating the benefits of NO \cdot while simultaneously mitigating its harmful effects.

H₂ protects against nitrosative stress

Nitric oxide reacts nearly instantaneously with superoxide (O₂ \cdot^-) to form pernicious peroxynitrite (ONOO \cdot), which is an extremely oxidative and cytotoxic molecule [37]. The reaction with O₂ \cdot^- directly lowers the availability of circulating NO \cdot , which further impairs mitochondrial function and muscle sarcomere contraction [37]. Thus, H₂ extends the circulating half-life of NO \cdot resulting in greater biological effects. The radicals, O₂ \cdot^- and NO \cdot , have important roles in actual exercise performance, such as mitochondrial ATP production and force of muscle contraction, and in mediating the benefits of exercise training. However, under excessive conditions, such as with intense and chronic exercise, these molecules contribute to decreased performance, impaired recovery and musculoskeletal injuries due to the formation of both ONOO \cdot and the extremely toxic hydroxyl radical (\cdot OH) [37]. Molecular hydrogen has been demonstrated to favorably regulate the production of both O₂ \cdot^- and NO \cdot by suppressing NADPH oxidase, which produces O₂ \cdot^- , and by

suppressing iNOS and nNOS while stimulating eNOS [1]. Additionally, H₂ can effectively reduce the toxic hydroxyl and peroxynitrite oxidants [3]. Importantly, H₂ prevented NO[•]-induced damage as evidenced by the elimination of nitrotyrosine levels, which was seen when NO[•] therapy was given alone [38].

H2 extends the half-life and potentiates the action of NO•

H₂ may facilitate the cycling of citrulline to arginine to nitric oxide by its effect on redox regulation by increasing GSH, CAT, SOD, GPx, etc. via activation of the Nrf2 pathway [36]. Byproducts of normal nitric oxide metabolism include inorganic nitrate and nitrite, which can be recycled and converted back to nitric oxide via certain redox-active enzymes. H₂ may favorably modulate this complex redox loop in facilitating the bioactivation of these oxyanions. Importantly, previous research has demonstrated that combination therapy of NO[•] and H₂ has a synergistic effect. The combination significantly attenuated lung neutrophil recruitment, inflammation, and premature cell death [38]. Similarly, the H₂ and NO[•] combination was more effective at reducing infarct size and improving left ventricular ejection fraction than when either was used separately [39]. Several of the benefits of a molecular hydrogen nitric oxide combination are summarized in Table 2.

Benefits of H2 and NO• combination		
Potentiate the bioactivity of NO•		
Increase levels of cGMP similar to actions of NO•		
Suppress toxic hyperstimulation of iNOS and nNOS		
Enhance activity of therapeutic eNOS, increased NO• production Increase hypoxia-induced NO• production		
Potentiate the citrulline-arginine regeneration		
Facilitate conversion of arginine to NO•		
Prolong ½ life of NO• by reducing O2-production		
Conservation of NO• metabolism via regulating its degradation		
Enhanced bioactivation of NO• metabolite products, nitrite/nitrate		
Reduce harmful nitrotyrosine levels induced by high NO• levels		
Reduce toxic ONOO- formation		

Table 2. Potentiating effects on NO[•] activity and metabolism

In an animal model of peripheral arterial disease, administration of H₂ increased capillarity density in the gastrocnemius muscle [40]. Nitric oxide exerts its effects by promoting the production of cGMP molecules. Intriguingly, H₂ enhanced cGMP levels nearly three-fold higher in ischemic hindlimbs [40], demonstrating that H₂ essentially i) acts as a NO[•] mimetic and ii) increases NO[•] bioactivity in muscles under low oxygen conditions [40], such as what happens during intense exercise. The previously discussed benefits of both H₂ and NO[•], both independently and collectively, provides added credence to the concept that their combination may provide significant benefits for exercise performance.

Methods and Results

The following results obtained from ingesting the functional beverage demonstrate its practical and significant benefits (*sections entitled "Increased nitric oxide production", "Blood flow" and "Improvement in aerobic capacity" are modified from a previous publication [36]*).

Increased nitric oxide production

The beverage significantly increased endogenous production of nitric oxide as shown in [Figure 1](#) and [Figure 2](#). The nitric oxide levels were increased from being depleted at baseline to the optimal level in 30 min, followed by a slight gradual trend upwards at hours 1, 3, 5, and 8 from baseline testing ([Figure 1](#)). Nitric oxide levels were increased to optimal levels for at least eight hours following product ingestion.

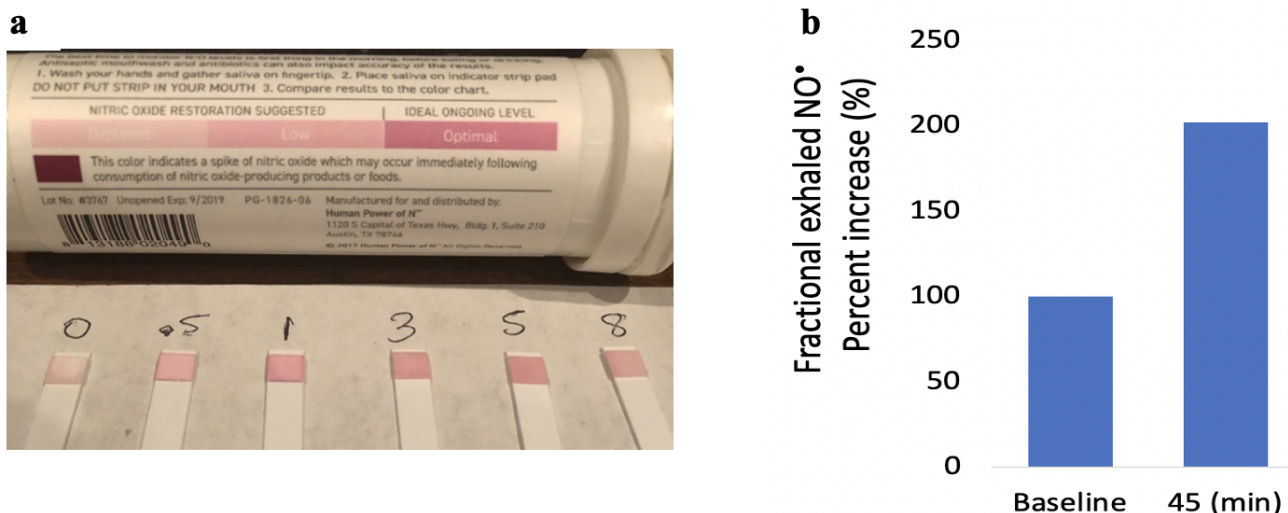


Figure 1. Increased nitric oxide production following ingestion of product. **(a)** Baseline (far left) depleted nitric oxide levels; 0.5, 1, 3, 5, and 8 h after ingestion optimal nitric oxide levels. **(b)** represents the average percent increase of fractional exhaled nitric oxide (FeNO[•]) by 12 subjects following 45 min after product ingestion compared to baseline.

Similarly, as shown in [Figure 2](#), using the FDA-approved NIOX VERO machine (Aerocrine AB, Solna, Sweden) the increased nitric oxide levels are also observed. NIOX VERO is a medical device that measures fractional exhaled nitric oxide (FeNO[•]), which is a commonly used to help diagnose and manage asthma in children and adults [41]. The baseline FeNO[•] of 12 subjects (six men and six women; age 45.3 ± 21.1) were obtained, and then they ingested H₂ Bev H₂-infused NO[•]-stimulating product and their FeNO[•] levels were measured again 45 min later. The product resulted in an average of 202.49% increase in FeNO[•] levels. Interestingly, when HRW was ingested alone, the NO[•] levels slightly decreased. This may be due to hydrogen's ability to influence the regulation of NO[•] production and decrease its overexpression. Asthmatic patients present with abnormally elevated NO[•] levels due to an overactive immune response. Molecular hydrogen has been demonstrated to decrease airway inflammation in allergic asthmatic mice [42]. Anecdotally, asthmatic consumers have reported beneficial relief from ingestion of molecular hydrogen but cite greater therapeutic effects from ingestion of the NO[•]-stimulating product. For example, a 12-year-old female reported an average Peak Flow Meter Reading at baseline of 220 mL/min. However, following 15 min of product ingestion, the flow meter increased by 15%. Finally, after three months of daily use, the baseline flow meter increased to 350 mL/min (160% increase).

Blood flow

As mentioned earlier, nitric oxide also promotes blood flow by inducing vasodilation. Optimal blood perfusion is critical for normal organ function, wound healing, and exercise performance. Blood flow provides oxygen, nutrients, hormones, signaling metabolites, etc. to the cells, and is necessary to remove harmful metabolic waste products. Similar to nitric oxide, blood flow also decreases with age [43], which may contribute to neurodegeneration, neuropathy [44], an impaired immune system [45], and decreased exercise performance and tolerance. In line with the previous data, ingestion of the product induced an increase in nitric oxide production. [Figure 2](#) illustrates the

average preliminary data (triplicate measurements) of blood flow following the ingestion of the product. The blood flow significantly increased from baseline at 15 minutes and remained elevated above baseline for over 15 h compared to control. The peak increase (231%) occurred approximately five hours after ingestion.

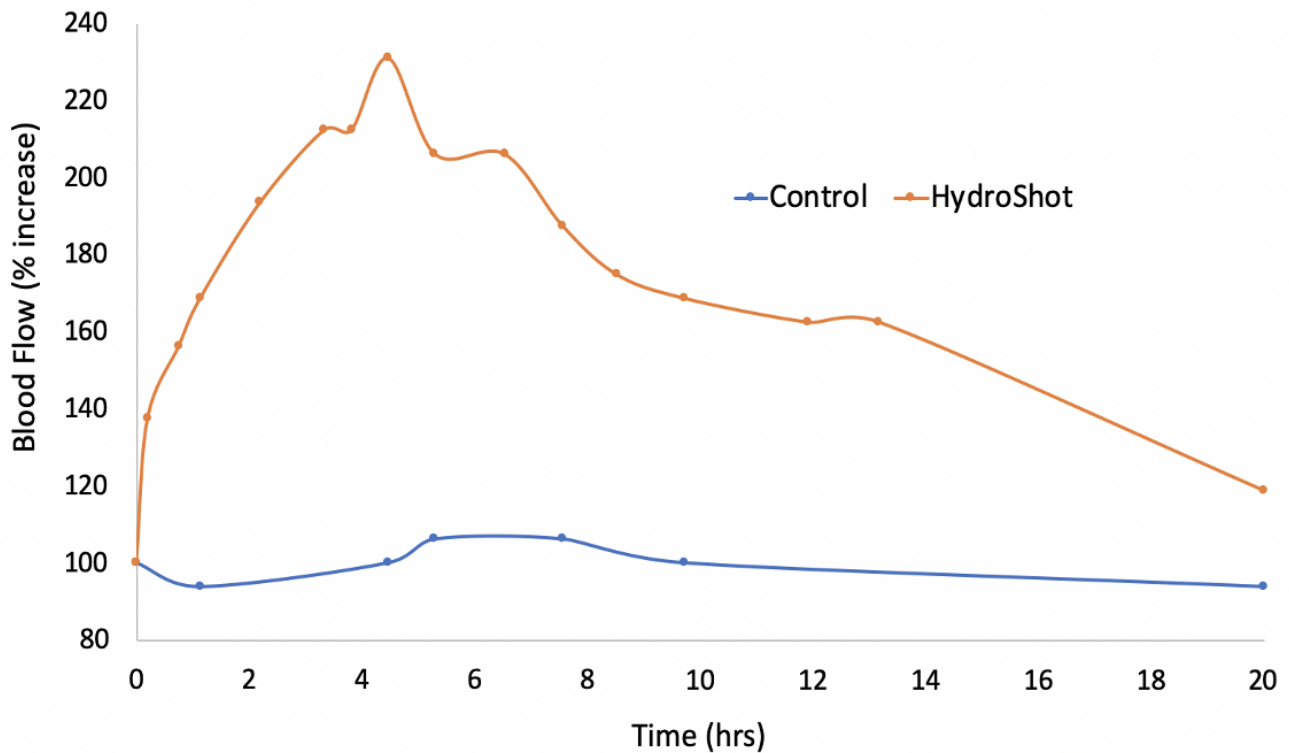


Figure 2. Changes in blood flow over 20 h following ingestion of beverage compared to its starting baseline and control. The functional beverage increased blood flow by 231% at five hours followed by a gradual decline towards baseline. Control values for blood flow did not significantly change compared to their starting baseline.

The increased blood flow was also reflected in thermal imaging of the hand as shown in [Figure 3](#). Baseline hand temperature averaged 96.8 degrees, and after 30 minutes it increased to 99.2 F.

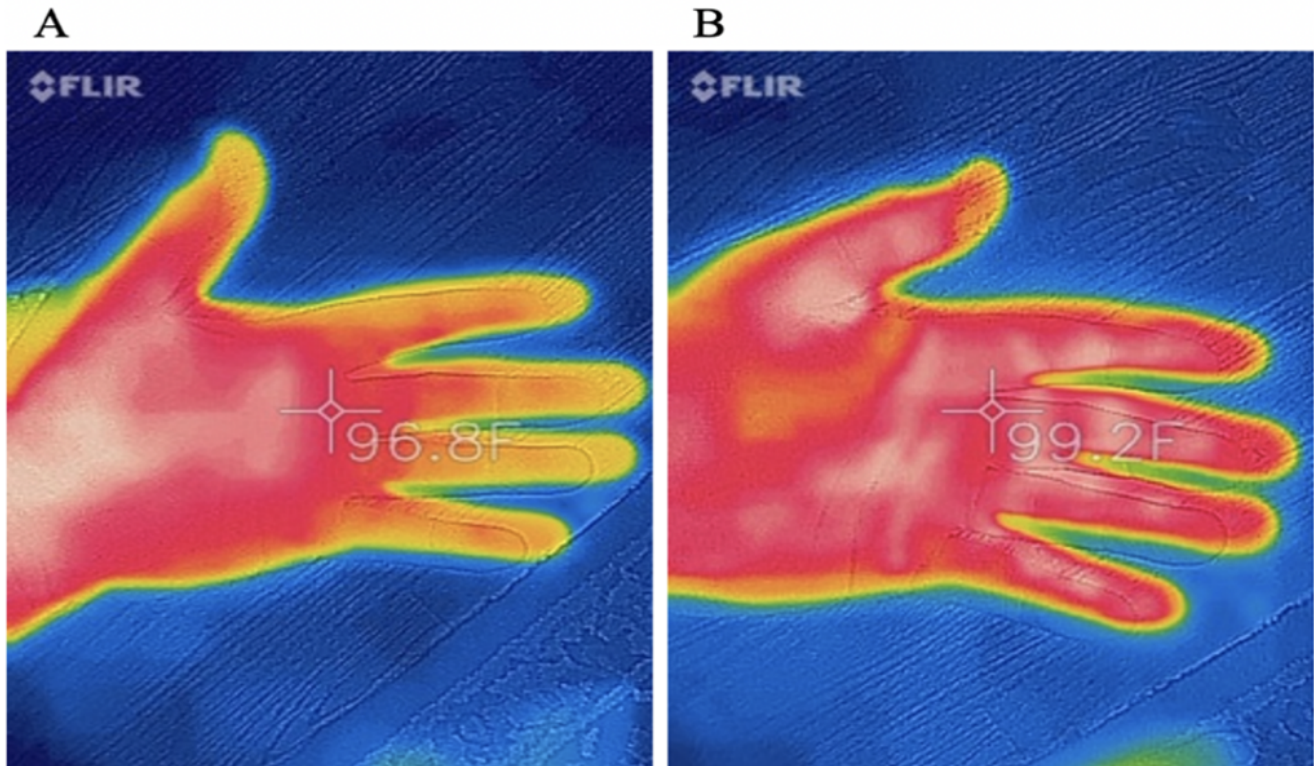


Figure 3. Thermal imaging of hand temperature following beverage ingestion. **(A)** Baseline temperature was 96.8 F 30 min later temperature increased to 99.2 F **(B)**.

The increased blood flow is likely due to a combination of molecular hydrogen and the other ingredients (e.g., citrulline) in the functional beverage. The increase in blood flow and the reflective thermal imaging was only mildly noticeable and for a shorter duration when either hydrogen alone or citrulline alone were ingested (data not shown). This may suggest additional synergistic effects between these two clinically relevant molecules.

improvement in aerobic capacity

Data was generated at Corpus Performance Facility in Dallas Texas. Testing was setup to determine the effects of the functional beverage on maximal aerobic power (MAP), functional threshold power (FTP), and maximal oxygen consumption (VO_2 max). Figure 4 illustrates the benefits of drinking the product compared to baseline.

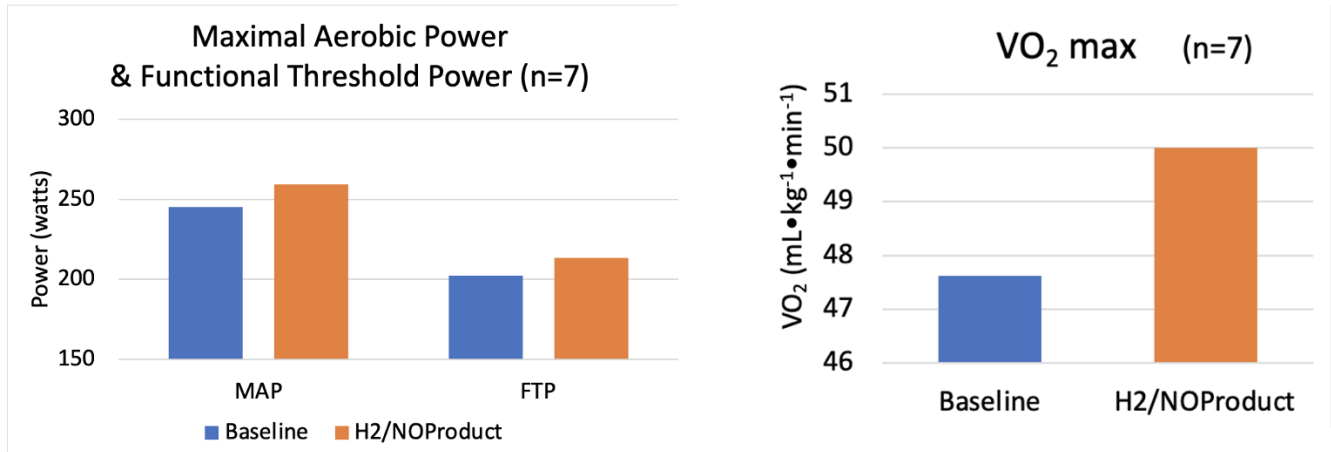


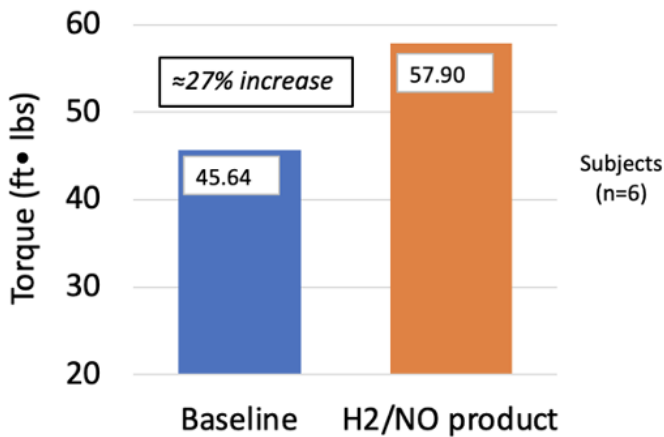
Figure 4. Changes in MAP, FTP and VO₂ max with and without the H₂/NO* product.

The functional beverage increased maximal aerobic power, functional threshold power, and VO₂ max (likely VO₂ peak). These results indicate that this H₂/NO* combination can improve exercise performance, which may in part be attributed to the increased nitric oxide production and blood flow.

Reduced fatigue and increased force production

Data was generated at the department of kinesiology at Southern Utah University. Testing was setup to determine the effects of the functional beverage on 50 maximal repetitions of isokinetic leg extension exercise. [Figure 5](#) illustrates the benefits of the H₂/NO* combination on fatigue and average force production.

a Average Torque (ft• lbs) for 50 leg extension repetitions at 180°•s⁻¹



b

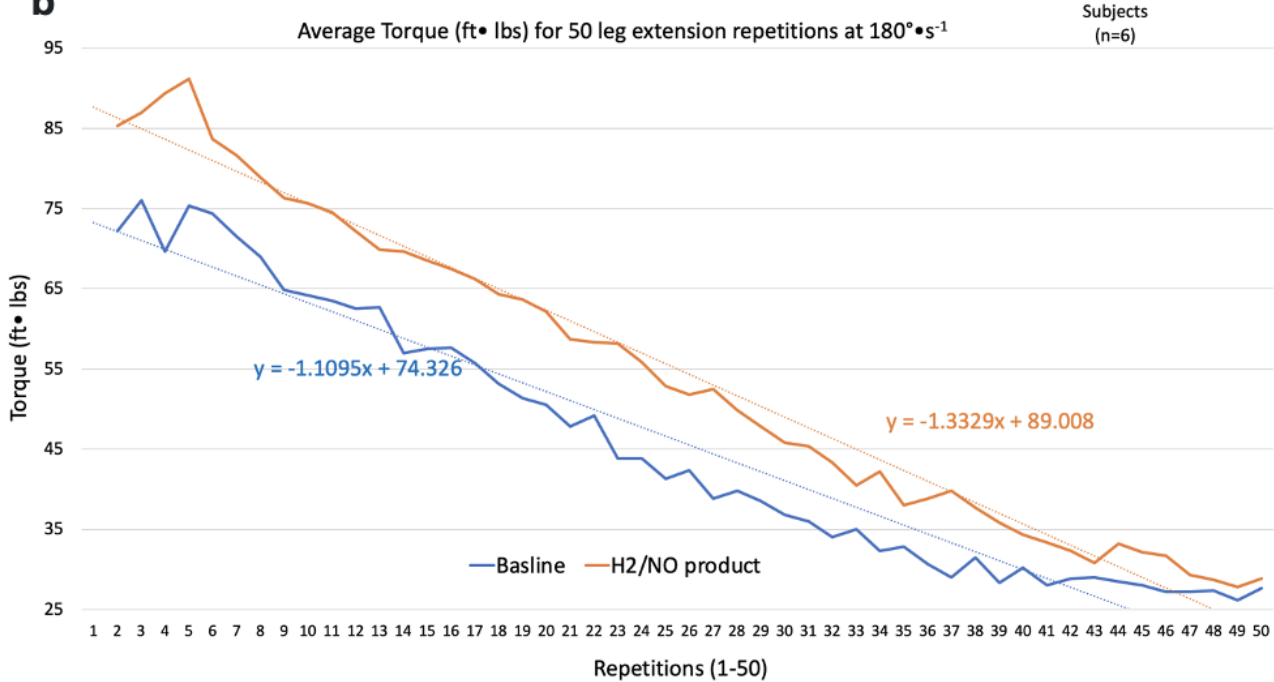


Figure 5. Effects of the beverage on average torque production in seven subjects. **(a)** Average torque of 50 repetitions with and without the beverage. **(b)** Average fatigue curve of subjects with and without the beverage. Equations of each slope are shown on graph. baseline (blue), beverage (orange).

The functional beverage significantly increased the average torque production during the 50 repetitions of leg extension by $\approx 27\%$. Additionally, as seen in [Figure 5 B](#), the average peak force was mainly increased during the initial repetitions. The greatest increase was 14.3 ft•lbs and the lowest increase was 1.3 ft•lbs. The first 28 repetitions were above 10 ft•lbs. These results indicate that the H₂/NO combination can significantly enhance force production and delay muscle fatigue.

Enhanced cognitive function

Study was conducted Corpus Performance Facility in Dallas Texas. Testing was setup to determine

the effects of the H₂/NO* combination on various indices of cognitive function including speed, accuracy, and focus. The somatosensory assessment was carried out using the scientifically validated Brain Gauge (Cortical Metrics, Chapel Hill, NC, USA.) [46]. Figure 7 illustrates the average significant benefits of the functional beverage on cognitive performance in eight subjects.

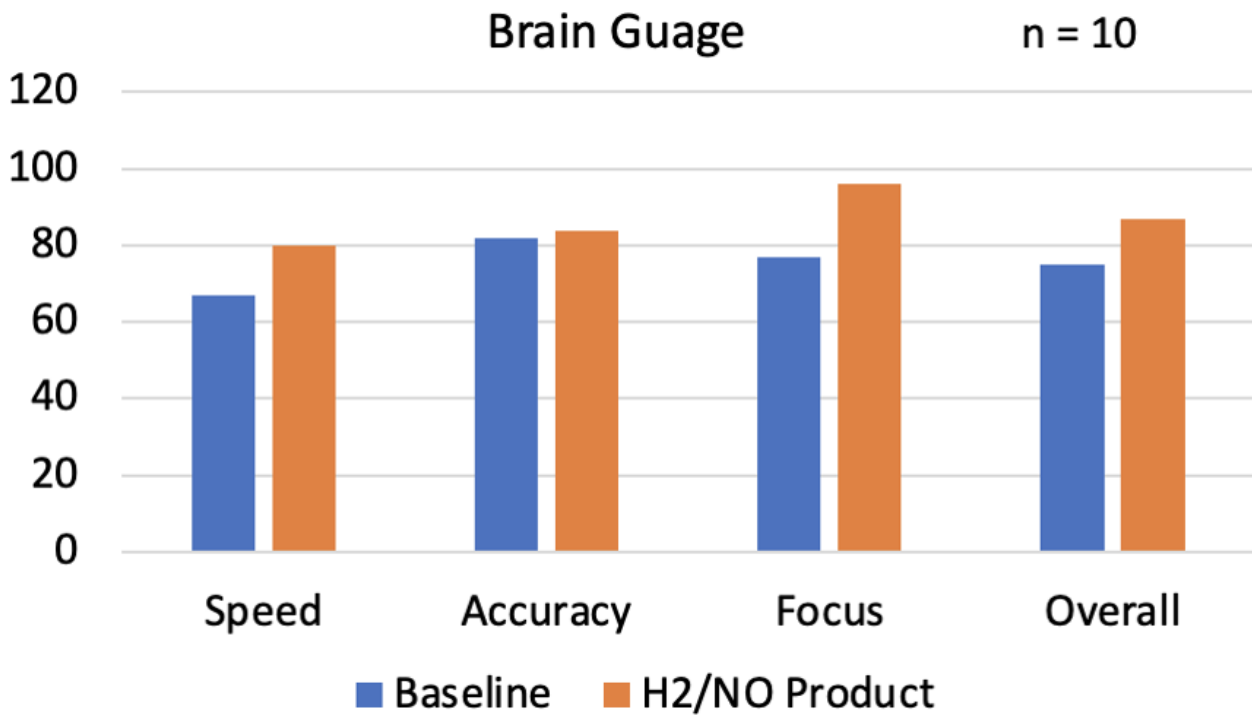


Figure 6. Clinical effects of the functional beverage on cognitive function

In all measured parameters except accuracy, the beverage increased the categories of cognitive function including, focus, and overall cognitive performance. Similar data were obtained in one 74-year-old subject who performed testing on multiple occasions to establish baseline and changes after 30 min of ingesting the functional beverage (See Figure 7).

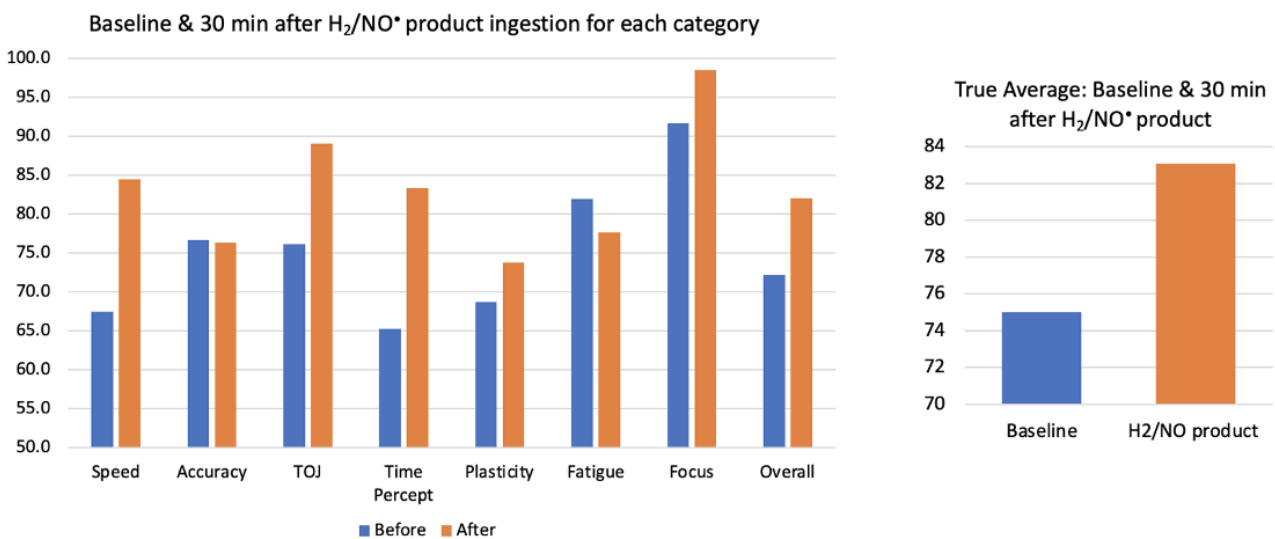


Figure 7. Changes in cognitive function in 74-year-old man following ingestion of beverage

Similar to subjects [Figure 6](#), the subject in [Figure 7](#) significantly improved in all indices of cognitive function except accuracy. Typically, when speed of reaction increases, accuracy goes down; however, the H₂/NO^{*} combination product led to significant increases in speed and still managed a trend in improving accuracy.

Discussion and Conclusion

These preliminary clinical results demonstrate that the combination of H₂ and nitric oxide has significant benefits in improving exercise and cognitive performance. This is attributed to the biological effects of both molecular hydrogen and nitric oxide. These gases likely act both independently and synergistically to enhance human performance. It is well established the optimal nitric oxide levels are critical for muscular and cognitive function. Optimal levels of nitric oxide result in optimal levels of blood perfusion into tissues resulting in maximal exercise capacity, recovery, and injury. However, like nitric oxide, blood flow also decreases with age (largely due to the declining nitric oxide levels). Nitric oxide has dual effects at mediating exercise function/benefits, and at mediating nitrosative damage/exercise intolerance. Molecular hydrogen can favorably regulate the biological activity of nitric oxide by extending and potentiating its beneficial effects, as well as mitigating against its harmful effects. These benefits are illustrated by the enhanced athletic and cognitive performance following the ingestion of the H₂-infused, nitric oxide-stimulating functional beverage. These benefits include increased nitric oxide production, increased blood flow, increased aerobic capacity and threshold, increased peak muscle force, decreased muscular fatigue, and enhanced cognitive function. It is concluded that Hydro Shot is uniquely qualified for sports performance and exercise medicine, and that additional mechanistic and clinical research is encouraged.

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