

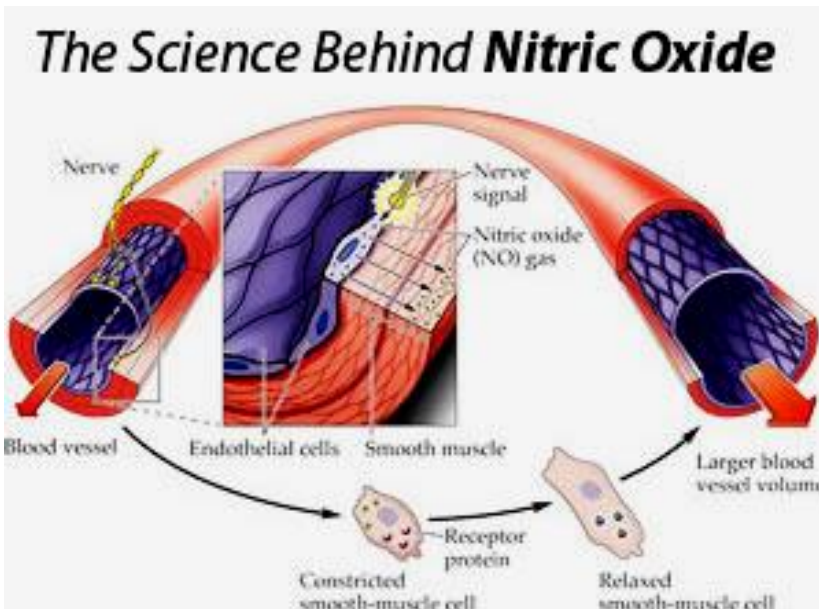
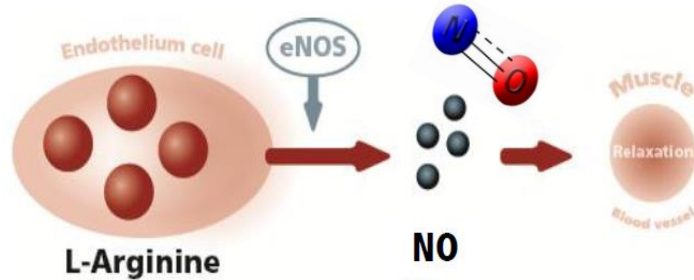
NITROFLOW PERFORMANCE² and RED BEET VINITROX – Mechanisms of Nitric Oxide (NO) Formation Targeting Maximised Athletic Performance

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Improved Endurance and Severe Short-Term Exercise with NO

Nitric oxide (NO) is a volatile, gaseous signal molecule, produced within the body. It is unique in its property to be formed within a cell, to penetrate cell membranes, and to regulate cell functions in other cells. NO is formed from the amino acid L-arginine by the enzyme system NOS (nitric oxide synthase).

NO has many functions in body tissues and systems, amongst others it plays an important role in vascular tone, blood pressure, muscle contraction, muscle growth and cell kinetics (reaction to contractile activity). NO is one of the main vaso-active mediators in the endothelial tissue. An increased NO formation causes a blood vessel dilatation (vasodilation, vasorelaxation), resulting in improved blood flow and enhanced microcirculation. The flat-formed, endothelial cells form the inner layer of blood vessels, and their most important function is the maintenance of an adjustable barrier between the blood vessel and the extravascular space. They play thereby a crucial role in the regulation of the vascular tone. An easing of tension in a blood vessel is linked with an enhanced vessel diameter, yielding an increased blood circulation.



Within the interested sports community, particularly in bodybuilding, an increased blood flow is considered identical with an enhanced "pump", i.e. the swelling of the muscles, which is mainly caused by exercise induced NO formation in the active muscles. There is a high reactivity of this process ("pump") to physical exercise. This "pump" is not only recognizable during activity, but stays for several hours. The increased blood flow after the activity is also beneficial towards a faster recovery. It is therefore not surprising that many athletes have looked at vasodilators to embellish their performance by improving blood flow and concomitant oxygen supply. And there is scientific evidence in support, indeed. The powerful performance increase by enhancing NO formation in the body has recently been shown for endurance as well as for high-intensity running bouts¹. The intake of 500 ml nitrate-rich beetroot juice (with converted 694 mg NO₃⁻) during 6 days increased nitrate values in the blood from day 4 on and reduced at the same time systolic blood pressure significantly (124 vs. 132 mmHg, p<0.01). The exercise-induced gain of oxygen demand of the lungs

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¹ Bailey et al, 2009: Dietary nitrate supplementation reduces the O₂ cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol*

was reduced by 19% during moderate exercise ($p < 0.05$). Meaning that with less oxygen the same performance could be maintained, or in conclusion, with the same amount of oxygen a higher performance would be possible. This outcome is accounted for the vasodilative effect of the formed NO in the body and the concomitant improved microcirculation and oxygen supply. On day 6 of that very study a time-to-exhaustion trial at a fixed severe work rate was performed, too. The time to task failure was extended by a massive 16% from 9 min 43 sec to 11 min 15 sec ($p < 0.05$) on average! Such impressive performance enhancements are even more remarkable, when thinking that the causal reduced oxygen cost by supplementation of nitrate-rich red beet juice cannot be acquired by means of long-term endurance training^{2,3}! Follow-up studies confirmed these performance improvements with supplemental beetroot^{4,5,6,7,8,9}. These results suggest that **NO induces an increased work load tolerance during endurance as well as during high-intensity anaerobic, lactic acid forming, sports!** The potential performance benefits are also recognized by renowned institutions such as the *Australian Institute of Sport*, which ranges beetroot as an A-supplement:

<http://www.ausport.gov.au/ais/nutrition/supplements/groupa>

Increased NO Formation with Nitrate from Vegetables and The Amino Acids Arginine, Ornithine and Citrulline

The formation of NO in the body is influenced by various factors, one of which is the increased intake of nitrate. Potentially, nitrate can improve mitochondrial function and thereby athletic performance. Mitochondria play a key part in the cellular metabolism. The well-known health benefits of vegetables may be partly explained by some of their positive effect on mitochondrial function. These effects of nitrate on mitochondria, as published in [Cell Metabolism](#), are of physiological interest insofar as it shows how nitrate can reduce oxygen consumption during physical exercise. The researchers could also



demonstrate that nitrate reduces blood pressure of healthy individuals. In fact, nowadays there are medical drugs based on nitrate (NO_3^-) or nitrite (NO_2^-) on prescription, because these substances are enzymatically metabolised to NO as mentioned above, resulting in a vasorelaxation and the concomitant blood pressure reduction. The research addresses also the use in other various health conditions, such as insufficient blood supply to tissues, stroke, sickle cell disease, and gastric diseases¹⁰.

Nitrate is a nutrient for plants and, in consequence, abundantly present in plants. Beetroot, spinach, green leafy salads and cabbages are particularly rich sources. Dietary NO_3^- is efficiently degraded by the bacteria of the mouth cavity to NO. Consequently, the consumption of nitrate-rich foods seems simple and cost-efficient to increase NO formation in our body. Unfortunately, it is not a very effective

² Burnley et al, 2000: Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. *J Appl Physiol*

³ Wilkerson et al, 2006: Influence of initial metabolic rate on pulmonary O_2 uptake on kinetics during severe intensity exercise. *Respir Physiol Neurobiol*

⁴ Lansley et al, 2011: Dietary nitrate supplementation reduces the O_2 cost of walking and running. *J Appl Physiol*

⁵ Cermak et al, 2012: Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *Int J Sport Nutr Exerc Metab*

⁶ Fulford et al, 2013: Influence of dietary nitrate supplementation on human skeletal muscle metabolism and force production during maximum voluntary contractions. *Muscle Physiol*

⁷ Wylie et al, 2013: Dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance. *J Appl Physiol*

⁸ Muggenridge et al, 2013: A single dose of beetroot juice enhances cycling performance in simulated altitude. *Med Sci Sports Exerc*

⁹ Haider et al, 2014: Nitrate supplementation enhances the contractile properties of human skeletal muscle. *Med Sci Sports Exerc*

¹⁰ Presley et al, 2010: Acute effect of a high nitrate diet on brain perfusion in older adults. *Nitric Oxide*.

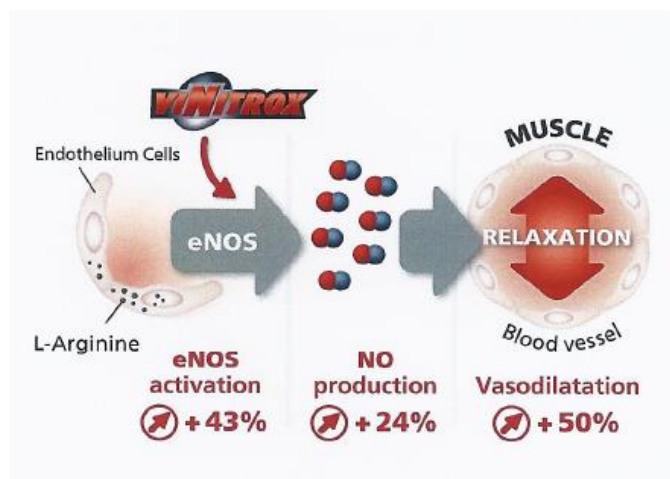
way since nitrate contents in vegetables are largely variable due to seasonal and cultivation-dependent reasons. Furthermore, assuming a very high nitrate content of 3000 mg per kg fresh vegetables, a quantity of 230 g would have to be ingested to reach common nitrate quantities of supplementation studies! Such volumes of fresh vegetables would influence dietary composition and may provoke gastrointestinal problems. However, such vegetables are suitable to produce concentrated, nitrate-rich food supplements from it. Said this, it is still necessary to analyse the nitrate content of each batch of fresh vegetables, and as a result thereof to adjust to the desired exact nitrate content in the finished product by dilution or concentration. This procedure only will allow to correct for biological variation of nitrate content and to manufacture a food supplement with a precise nitrate content.

Similarly as nitrate, the two amino acids L-arginine and L-ornithine serve as substrate and precursors to the formation of NO in our body. This pathway is well described, but its relevance in a living organism is not established. Nevertheless, many supplements claiming to increase NO formation are virtually only formulated with these two amino acids. Another amino acid, L-citrulline, can be metabolised to L-arginine. And as a matter of fact, there is evidence that the ingestion of L-citrulline is increasing blood levels of arginine to a greater extent than the direct ingestion of L-arginine itself¹¹. Increased plasma arginine levels and improved performance after several days of L-citrulline supplementation have been found in another recent study¹².

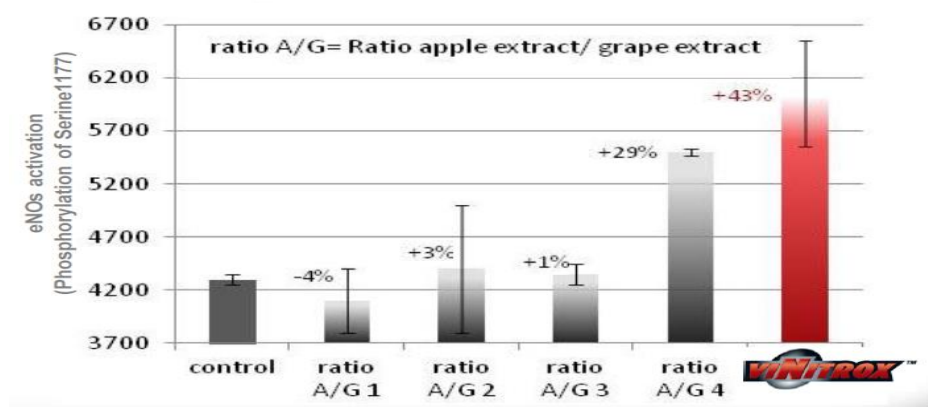
Boosting the NO Enzyme System with Polyphenols and Peptides

A promising mechanism of optimising NO formation is the use of enzymes and/or targeting the efficiency of involved enzyme systems. A functional ingredient in this field is VINITROX™, a blend of extracts from grape and apples, which targets the facilitation of the enzyme endothelial NO synthase (eNOS). The polyphenols of VINITROX™ work synergistically: the combination and the specific ratio of apple and grape polyphenols work better than single administration (1+1 = 3).

A first *in vitro* study with VINITROX™ demonstrated this synergistic effect with a 43% increase in eNOS activation, resulting in 24% and 50% higher NO synthesis and vasodilatation, respectively. The potential ergogenic benefits on performance were confirmed in a large human study with 48 subjects, where time-to-exhaustion was increased by +9.7% on a cycling ergometer¹³.



In vitro study: Effect of ViNitrox on the activation of eNOs

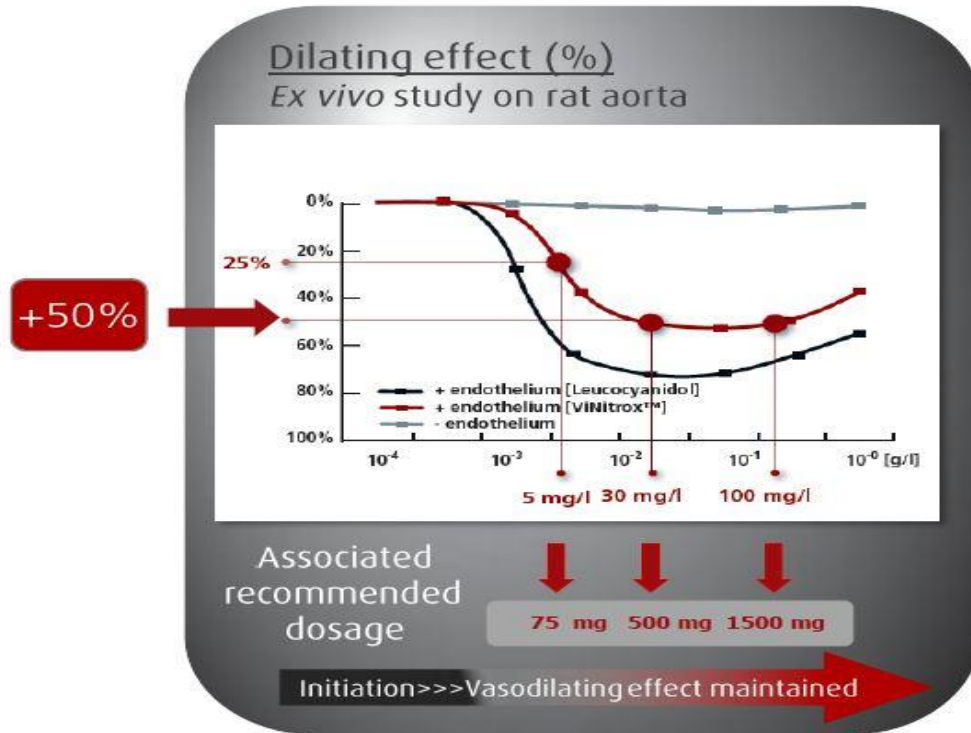


¹¹ Schwedhelm et al, 2008: Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. Br J Clin Pharmacol.

¹² Suzuki et al, 2016: Oral L-citrulline supplementation enhances cycling time trial performance in healthy trained men: Double-blind randomized placebo-controlled 2-way crossover study. J Int Soc Sports Nutr

¹³ Deley et al, 2017: An acute dose of specific grape and apple polyphenols improves endurance performance: a randomized, crossover, double-blind versus placebo controlled study. Nutrients

Especially impressive is the comparison with a medical drug (Leucocyanidol), which showed a similar efficacy of VINITROX™ in an earlier *ex vivo* study. Notably, a dosage of between 200 to 500 mg VINITROX™ seems most efficacious and increasing dosage does not result in a greater dilating effect.

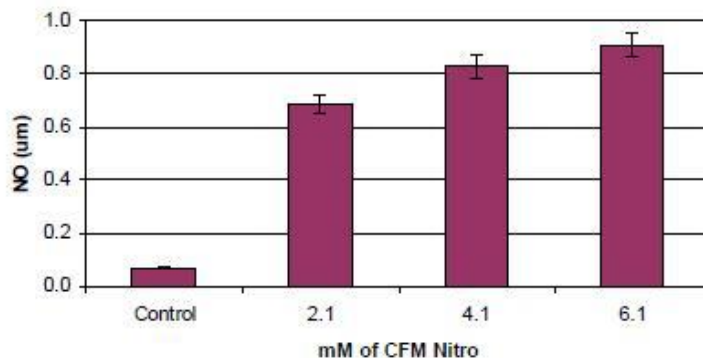


Another natural ingredient targeting intravascular NO formation is called CFM® Nitro, a fractionated whey protein with bioactive peptides and proteins, which bind to endothelial tissue thereby protecting the inherent stimuli of NO formation. As a result, the eNOS system is promoted in its efficiency.

CFM® Nitro was developed by molecule size fractionation and testing of various fractions on their specific effect on NO synthesis. It was already previously known that two milk peptide fractions of different molecular size facilitate NO formation. In order to differentiate and validate the efficacy and the specific role of high- and low-molecular size peptide fractions, their individual and combined effect on human endothelial cells of the lung aorta was examined (*ex vivo*).

Both fractions increased NO synthesis in endothelial cells up to a factor 9 in the examined dosage. CFM® Nitro, containing low- and high-molecular peptide fractions, increased NO formation by a factor 9.5 to 12.7!

FIGURE 3: Effect of CFM® Nitro on NO Production in HPAE-26 Cells



Antioxidative Cell Protection Improves Performance

In recent years, it was discovered that – in contrast to previous positive expectations – high intake of isolated antioxidants such as vitamin C or beta-carotene may impair physical performance or even health. For this reason, high-dosage mono-substance supplementation is nowadays discouraged, as far as it is not about short-term intake with acute high oxidative stress situations. Chronic high ingestion impairs training-induced adaptation of the body to oxidative stress. However, in contrast to vitamin C certain plant extracts, each comprising hundreds and thousands of antioxidative substances, seem to improve performance and recovery of athletes without compromising training adaptations and health. Polyphenols protect from various oxidative stress, have anti-inflammatory function, and enhance microcirculation through their dilatative effect, all of which ultimately improves performance and recovery¹⁴.

In fact, from very recently it is now not anymore just "oxidative stress", but also "nitrosative stress" and integrally spoken of redox homeostasis and redox regulation. Science concentrated fairly long only on peroxy radicals, but the four other main free radical types are superoxide anions, hydroxyl-, peroxynitrite-, and singlet oxygen radicals. Each type of radical is neutralised by other antioxidants, highlighting the importance of a diet rich in various (multi-coloured) vegetables and fruits.



Based on this rationale **SPECTRA™** was developed, a blend of plant concentrates and extracts, combining a spectrum of 29 fruits, vegetables and herbs. Its efficacy of a single dose on total radical modulation was examined in a human study. The outcome showed a clear reduction during 3 hours post ingestion. The same study revealed also a significant reduction in the inflammatory marker TNFalpha (*ex vivo*)¹⁵.

As to expect also here an increase of NO could be measured along with, particularly interesting for athletes, an increased mitochondrial and cellular oxygen consumption with concomitant lower blood glucose levels, which indicates higher fat oxidation for energy production.

Spectra™ Reduces Free Radical Production for 3 Hours after Ingestion

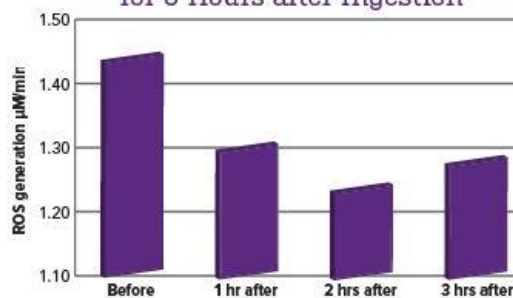


Figure 1.

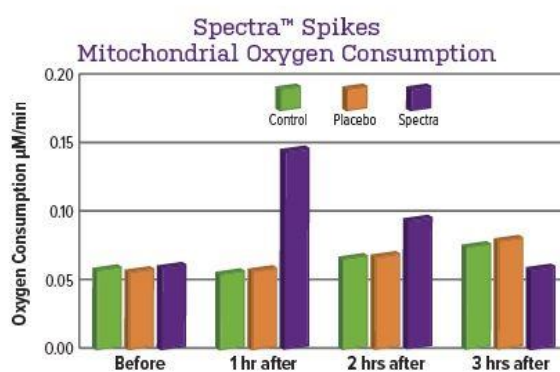


Figure 4.

Spectra™ Naturally Increases Nitric Oxide Levels by 64%

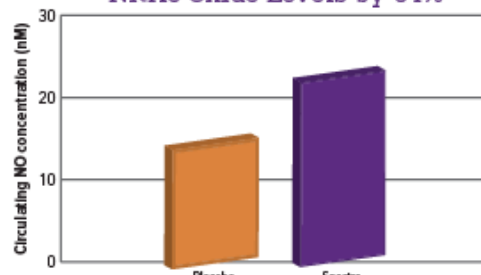


Figure 2.

In accordance with these results a study with **NEW ZEALAND BLACKCURRANT** revealed an increased fat oxidation as well as improved endurance performance. 7 days' supplementation with NZ blackcurrant resulted in a 27% increase in lipid oxidation at 65% VO²max, a 2.4% improvement (time

¹⁴ Braakhuis et al, 2014: Effects of dietary antioxidants on training and performance in female runners. Eur J Spor Sci

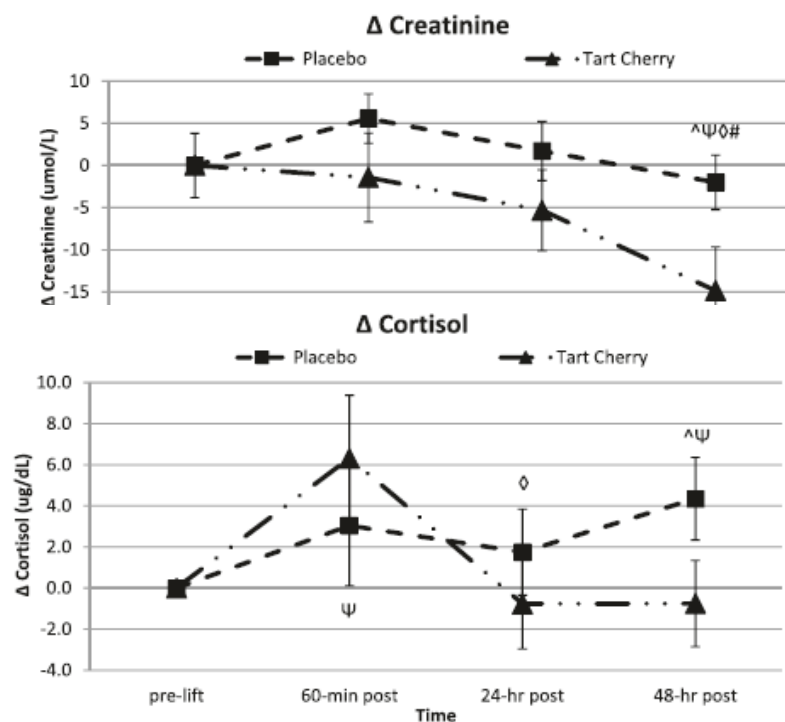
¹⁵ Nemzer et al, 2014: New insights on effects of a dietary supplement on oxidative and nitrosative stress in humans. Food Sci Nutr.

reduction) in cycling performance, and an accelerated lactate clearance within 20 minutes after exercise¹⁶. Related to a total run time of 28 minutes, 2.4% translate to a time reduction of more than 30 seconds. This study has been replicated, yet not published yet as of September 2017, demonstrating an even greater improvement, i.e. a cycling time trial reduction of 3.6%.

New Zealand blackcurrants contain very high contents of polyphenols grace to the climatic situation. Particularly so-called anthocyanins, flavanols and flavonols, which are considered responsible for the vasorelaxative/dilatative effect of NZ blackcurrant. The same hypothesis applies insofar as anthocyanins have the potential to increase NO formation in endothelial cells, as well as to diminish free radical-induced NO reduction. Improved blood flow through increased NO formation, and the concomitant increased oxygen supply to muscles is relevant for many sports. Not solely for endurance performance, where improved lipid oxidation by enhanced oxygen consumption and subsequent sparing of glycogen stores is crucial. Recovery time is also shortened by improved microcirculation, resulting in e.g. accelerated lactate clearance.



Even better evidence is available for the supplementation with **MONTMORENCY TART CHERRY**, like NZ blackcurrant rich in ergogenic anthocyanins. Several studies demonstrated beneficial effects on endurance performance^{17,18}, as well as on fastened recovery^{19,20}. Improved recovery was confirmed in another study with strength athletes, demonstrating a reduced strength loss and lower catabolic stress markers such as cortisol, creatinine, creatine kinase, ureic acid, alanine- and aspartate aminotransferase within 48 h after resistance exercise²¹. This outcome points to a reduced muscle damage and fastened recovery. An attenuated muscular damage, and, in analogy to NZ blackcurrant, improved aerobic performance was also found in endurance athletes¹⁷. The positive evidence of Montmorency tart cherry persuaded the *Australian Institute of Sport* to list it as a B-Supplement²².



¹⁶ Cook et al, 2015: New Zealand blackcurrant extract improves cycling performance and fat oxidation in cyclists. Eur J Appl Phys.

¹⁷ Levers et al, 2016: Effects of powdered Montmorency tart cherry supplementation on acute endurance exercise performance in aerobically trained individuals. J Int Sports Nutr.

¹⁸ Bell et al, 2014: Montmorency cherries reduce the oxidative stress and inflammatory responses to repeated days high-intensity stochastic cycling. Nutrients.

¹⁹ Bell et al, 2015: Recovery facilitation with Montmorency cherries following high-intensity, metabolically challenging exercise. Appl Physiol Nutr Metab.

²⁰ Bell et al, 2016: The effects of Montmorency Tart Cherry Concentrate Supplementation on Recovery Following Prolonged Intermittent Exercise. Nutrients.

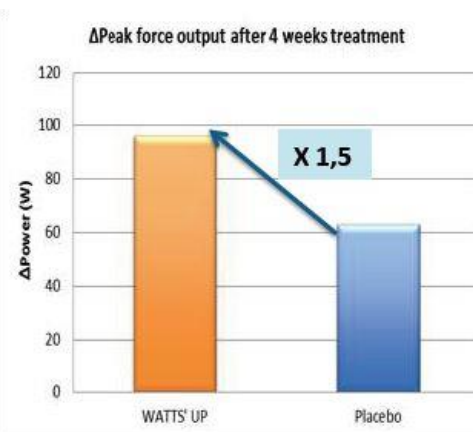
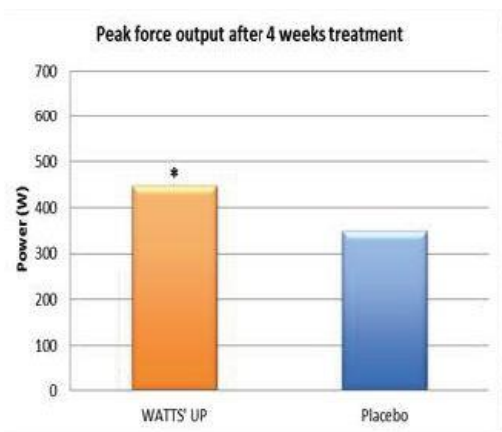
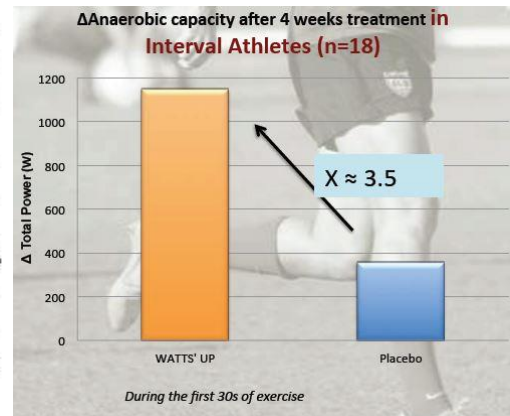
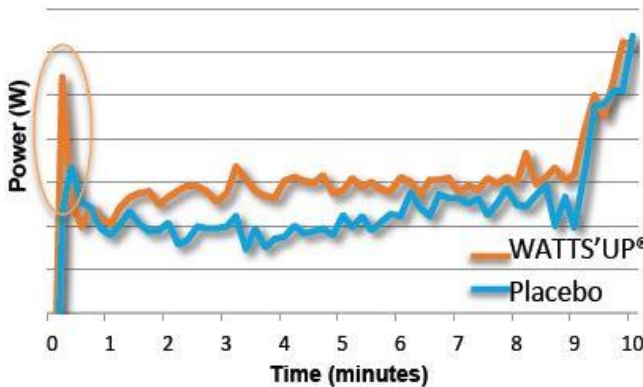
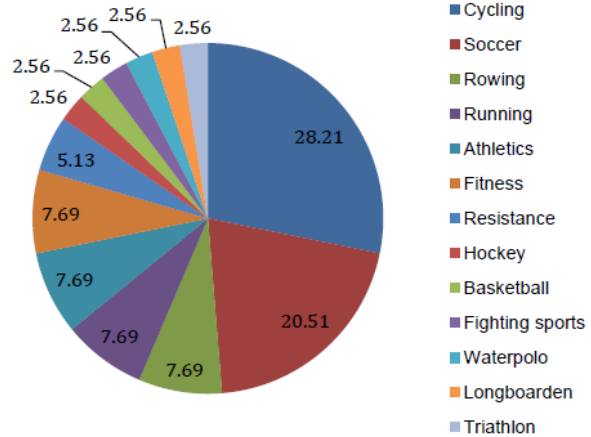
²¹ Levers et al, 2015: Effects of powdered Montmorency tart cherry supplementation on an acute bout of intense lower body strength exercise in resistance trained males. J Int Soc Sports Nutr.

²² http://www.ausport.gov.au/ais/sports_nutrition/supplements/groupb, 29-03-2017

Also bioflavanoids from citrus fruits have the potential improve performance and accelerate recovery. A pre-clinical trial on endurance athletes of various disciplines with the citrus fruits extract WATTS'UP® found improvements of +5% in total power and +36% in peak power. The largely increased peak power is particularly interesting for athletes involved in interval, strength and power sports. Besides, in a sub-group of interval sports athletes their anaerobic energy capacity was increase by factor 3.5!

WATTS'UP®

In consequence, the reduced oxygen consumption per generated watt (power) with the supplementation of WATTS'UP® indicates not only an improved oxygen supply grace to an increased microcirculation, but also to a more efficient oxygen use and energy (ATP) production in cell's "power plants", the mitochondria. As a result, higher average and maximum watt performance could be measured during the exercise bout. Not unexpectedly a more accentuated effect was found in moderately (<6 h/week) compared to more intensively (>6 h/week) training athletes. However, the reason for the chosen supplementation duration of 4 weeks remains unclear since other studies with antioxidative plant extracts usually chose shorter interventions.

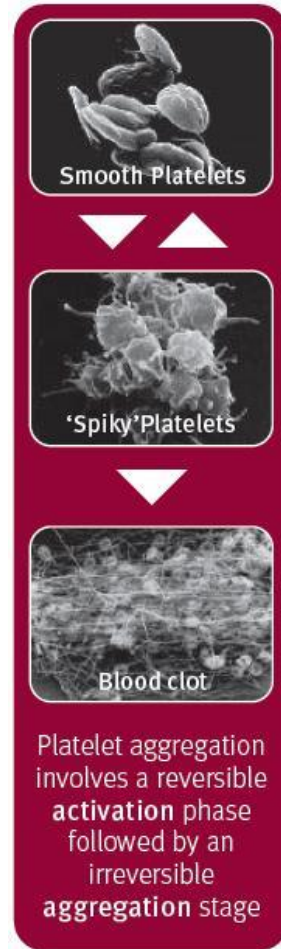




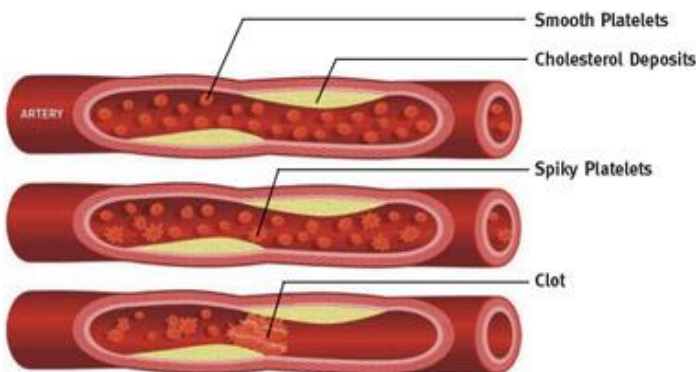
Improved Blood Flow by Maintaining Normal Blood Platelet Aggregation with Tomato Extract

Improved microcirculation, oxygen and nutrient supply is not reduced to simply increasing NO formation. Due to various factors in daily life such as stress, sport or high blood cholesterol, blood platelets can become activated and form so-called "spikes", scientifically named pseudopodia. In this state blood platelets can aggregate, get hooked on the endothelial wall and form a clot (thrombus). Normally, blood platelets are soft with a smooth surface, allowing the blood to flow lissom through the vessels. In athletes an increased blood platelet aggregation may occur caused by dehydration. Fruitflow® maintains the surface of blood platelets smooth and flexible, which inhibits excessive aggregation in the vessels. Notably, normal blood coagulation is not hindered, which is important with regards to bleeding injuries.

Fruitflow® is gained from tomato extract and proven to maintain normal platelet aggregation and improve blood flow in 10 human clinical studies. A respective health claim has been officially authorised by the European Food Safety Agency EFSA. The ergogenic effect shows within 1.5 hours after intake and remains for about 12 to 18 hours. The maximal effect is reached between 3 to 6 hours after intake. Daily intake results in a permanent maintenance of efficacy.

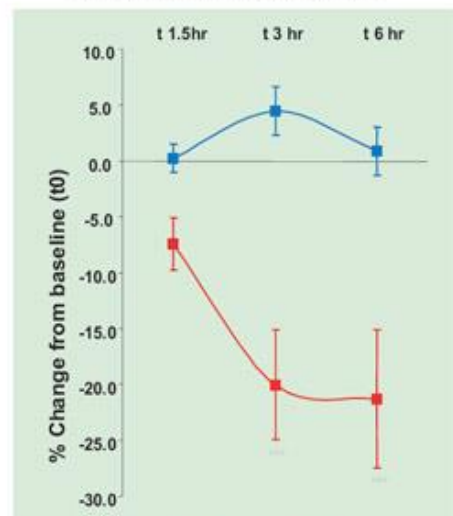


Platelet aggregation involves a reversible activation phase followed by an irreversible aggregation stage



Platelets stay smooth with Fruitflow® to support healthy blood flow.

Onset and duration of *in vivo* blood circulation effect



n = 23 ■ Control ■ Tomato extract

O'Kennedy et al. 2006a, AJCN

Comparison of NITROFLOW PERFORMANCE² and RED BEET VINITROX

Maximising NO synthesis with several, also synergistically working mechanisms, in combination with the maintenance of normal blood flow, offers a unique dietetic performance and recovery support for ambitious athletes. SPONSER® has two products in this area of functional supplements.

RED BEET VINITROX is a ready-to-drink flask with beetroot concentrate, providing 400 mg of naturally contained nitrate (NO₃⁻), corresponding to about the quantity of 500 ml fresh juice. Besides NO₃⁻ as precursor and substrate of NO formation, it provides several other ingredients to facilitate NO synthesis: ViNitrox®, CFM® Nitro, the two amino acids L-arginine and L-ornithine, as well as the patented tomato extract Fruitflow™ in the needed dosage to maintain smooth blood flow and inhibit excessive blood platelet aggregation.

Use instructions: *Minimally, take 1 flask about 3 hours pre-start, ideally another flask the evening or 6 hours before, depending on your starting time. In some studies intake was even advised during 4-6 days pre-race.*



NITROFLOW PERFORMANCE² is intentionally formulated without nitrate as a NO precursor. In contrast, it comprises a very innovative combination of multiple and various polyphenol-rich plant extracts, such as Montmorency tart cherry and New Zealand blackcurrant, both of which already several times clinically tested and proven on athletes. The incorporated plant extracts provide the body with precious polyphenols, whose antioxidative function protects cells during exercise and activate NO synthesis. The formulation is completed with L-citrulline and Fruitflow™.




Use instructions: *1 sachet daily during about 5 days, about 3 h pre-start, and optimally 1 to 2 days after race.*



In praxis, both products can be combined for maximal efficacy. However, if gastrointestinal problems occur with the concentrated beetroot juice, the ingestion of RED BEET VINITROX at 3 hours pre-start should be omitted. Instead, try to take 2 times 1 flask on the pre-race day.

Obviously, in **strength sports** the acute intake makes most sense for both products, prior to resistance exercise. This corresponds usually in several times' intake during a week.

As outlined in the article the products should not be taken permanently, but purposefully prior to competitions or during intense and wasting training weeks. This proceeding will support your performance as well as recovery, without affecting training-induced stress adaptations of the body.

| | Nitroflow Performance² (7 g, powder sachet) | Red Beet Vinitrox (60 ml flask) |
|---|--|---|
| Natural NO ₃ ⁻ from beetroot | | 400 mg |
|  | 150 mg | 3000 mg |
|  | | 400 mg |
|  | 250 mg | 200 mg |
| L-arginine | - | 500 mg |
| L-ornithine | - | 500 mg |
| L-citrulline | 1000 mg | |
|  | 250 mg | |
|  | 50 mg | |
| Oxi-fend[®] NZ Blackcurrant | 150 mg | |
|  | 250 mg | |
| Use instructions |  <p>Endurance-/Interval sports</p> <p>Daily for 4-5 days pre-race + 3 h pre-start + 1-2 days afterwards</p>  <p>Strenght sports</p> <p>1-2 h prior to training/competition</p> |  <p>Endurance-/Interval sports</p> <p>Pre-race evening + 3 h pre-start (ideally to be taken already 4-5 day pre-race)</p>  <p>Strength sports</p> <p>2-3 h prior to training/competition</p> |