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(54) PERFORMANCE ENHANCING COMPOSITION AND USE THEREOF

LEISTUNGSERHÖHENDE ZUSAMMENSETZUNG UND IHRE VERWENDUNG

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Description

Field of the invention

[0001] The present invention relates to the field of performance enhancing nutritional foods and food supplements, liquid and solid edible products such as sport drinks, energy drinks and energy bars.

Background

[0002] It is currently believed that improved sports performance can be attained by the intake of so-called sport drinks. These are usually non-carbonated and frequently contain fructose or other sugars, and complex carbohydrates, which are easily absorbed by the body, and are designed to promote the availability of energy and/or prevent or treat mild dehydration. Sport drinks also contain electrolytes (mainly sodium and potassium salts) and nutrients (proteins and amino acids). Sport drinks, energy drinks and other liquid, semi-solid and solid products, while marketed for athletes, are also consumed by non-athletes, as a snack, in situations where extra energy and endurance is desired.

[0003] Sometimes a distinction is made between sport drinks and energy drinks, the former tending to be more isotonic, and the latter containing more sugar and frequently also caffeine. In this context, no such distinction is intended, and the term "performance enhancing food or food supplement" includes sport drinks and energy drinks, as well as other liquid, semi-solid or solid forms, such as energy bars, tablets etc. as described in further detail below.

[0004] Physiological adaptation to exercise however involves major cardiovascular and metabolic changes. Oxygen consumption increases dramatically in the active muscles with a parallel increase in muscle blood flow. In these processes the endogenous gas nitric oxide (NO) plays an important regulatory role. NO increases blood flow to the muscles and modulates muscular contraction and glucose uptake (for review see STAMLER, J S, et al. Physiology of nitric oxide in skeletal muscle. Physiol Rev. 2001, vol.81, no.1, p.209-37).

[0005] In addition, NO is involved in control of cellular respiration through interaction with enzymes of the mitochondrial respiratory chain (for review see MONCADA, S, et al. Does nitric oxide modulate mitochondrial energy generation and apoptosis?. Nat Rev Mol Cell Biol. 2002, vol.3, no.3, p.214-20).

[0006] *In vitro* studies published in the 1990s show that NO is a modulator of mitochondrial respiration via reversible inhibition of cytochrome c oxidase (CARR, G J, et al. Nitric oxide formed by nitrite reductase of Paracoccus denitrificans is sufficiently stable to inhibit cytochrome oxidase activity and is reduced by its reductase under aerobic conditions. Biochim Biophys Acta. 15 May 1990, vol. 1017, no.1, p.57-62.; BOLANOS, J P, et al. Nitric oxide-mediated inhibition of the mitochondrial respiratory chain in cultured astrocytes. J Neurochem. 1994, vol.63, no.2, p.910-6; BROWN, G C, et al. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett. 19 Dec 1994, vol.356, no.2-3, p.295-8; CLEETER, M W, et al. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. FEBS Lett. 23 May 1994, vol.345, no.1, p.50-4; and SCHWEIZER, M, et al. Nitric oxide potently and reversibly deenergizes mitochondria at low oxygen tension. Biochem Biophys Res Comm. 1994, no.204, p.169-75).

[0007] NO may also interact at other sites of the mitochondrial respiratory chain and in the Krebs cycle (for review see Moncada, *supra*). While this important action of NO has been very well characterised in cell cultures, less is known about its physiological relevance *in vivo* and the effects of NO on cellular respiration during physical exercise. Shen and colleagues showed that administration of **NOS**-inhibitors *in vivo* during submaximal exercise leads to increased oxygen consumption in dogs (SHEN, W, et al. Role of NO in the regulation of oxygen consumption in conscious dogs. Circulation Res. 1999, no.84, p.840-5) and Lacerda and colleagues showed similar results in rats (LACERDA, A C R, et al. Evidence that brain nitric oxide inhibition increases metabolic cost of exercise, reducing running performance in rats. Neuroscience Letters. 2006, no.393, p.260-3). The majority of studies have been done using **NOS-**inhibitors while the effects of administering exogenous NO on exercise are largely unknown. In addition, studies in healthy humans are scarce.

[0008] The classical means by which NO production occurs is the L-arginine pathway, where NO is synthesized by specific enzymes, the NO-synthases. A fundamentally different alternative way of generating NO has been described more recently (LUNDBERG, J O, et al. Intragastric nitric oxide production in humans: measurements in expelled air. Gut. 1994, vol.35, no.11, p. 1543-6; BENJAMIN, N, et al. Stomach NO synthesis. Nature. 7 Apr 1994, vol.368, no.6471, p.502; ZWEIER, J L, et al. Enzyme-independent formation of nitric oxide in biological tissues. Nat Med. 1995, vol.1, no.8, p.804-9; and WEITZBERG, E, et al. Nonenzymatic nitric oxide production in humans. NO Biol Chem. 1998, no.2, p.1-7). In this NOS-independent pathway the inorganic anions nitrate (NO_3^-) and nitrite (NO_2^-) are reduced *in vivo* to form NO. Dietary nitrate (found mainly in green leafy vegetables) (MCKNIGHT, G M. Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. Gut. 1997, no.40, p.211-214; and Weitzberg, 1998, supra) is absorbed from the circulation by the salivary glands, secreted in saliva and partly converted to nitrite in the oral cavity by nitrate reducing bacteria. Swallowed nitrite can then enter the systemic circulation. Indeed, a recent study shows that ingestion of nitrate results in a sustained increase in circulating nitrite levels (LUNDBERG, J O, et

al. Inorganic nitrate is a possible source for systemic generation of nitric oxide. Free Rad Bio Med. 2004, vol.37, no.3, p.395-400). Further reduction of nitrite into bioactive NO can occur spontaneously in acidic or reducing environments (Benjamin *et al.* 1994, supra, Lundberg *et al.* 1994, supra) but is also greatly enhanced by various proteins and enzymes including deoxyhemoglobin in blood (COSBY, K, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med. 2003, vol.9, no.12, p.1498-505), deoxymyoglobin (SHIVA, S, et al. Deoxymyoglobin is a Nitrite Reductase That Generates Nitric Oxide and Regulates Mitochondrial Respiration. Circ Res. 9 Feb 2007), xanthine oxidase (MILLAR, T M, et al. Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions. FEBS Lett. 8 May 1998, vol.427, no.2, p.225-8) and possibly by enzymes of the mitochondrial respiratory chain (for review see LUNDBERG, J O, et al. Nitrate, bacteria and human health. Nat Rev Microbiol. 2004, vol.2, no.7, p.593-602; LUNDBERG, J O, et al. NO generation from nitrite and its role in vascular control. Arterioscler Thromb Vasc Biol. 2005, vol.25, no.5, p.915-22; and GLADWIN, M T, et al. The emerging biology of the nitrite anion. Nat Chem Biol. 2005, vol.1, no.6, p.308-14). NOS-independent NO production seems to complement the endogenous NO production especially during ischemia and acidosis when oxygen availability is low and the NO synthases operate poorly (Zweier *et al.* 1995, supra; Weitzberg *et al,* 1998, supra; DURANSKI, M R, et al. Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver. J Clin Invest 2005, vol.115, no.5, p.1232-40; Lundberg *et al,* 2004, supra). Tissue acidosis and relative hypoxia is present also during physical exercise and in this metabolic state, bioactivation of nitrite is likely enhanced.

[0009] The available information on the role of NO in healthy subjects and in particular in athletes during work or exercise is both insufficient and contradictory. Interestingly, the marketing of some currently available food supplements for athletes and bodybuilders refer to the vasodilatory effect of NO. One example is "NOX2" (Bodyonics, Ltd., USA), a product said to contain arginine alpha-ketoglutarate (A-AKG) and arginine-ketoisocaproate (A-KIC) and allegedly capable of boosting short term nitric oxide levels. Other products contain L-arginine, from which NO is synthesized by the NOS enzymes, and the beneficial effects of NO are often referred to, however without offering more detailed explanations.

[0010] The relation between peak work rate and resting levels of nitrate in plasma and urine from subjects with different levels of physical fitness has been studied (Jungersten et al., Both physical fitness and acute exercise regulate nitric oxide formation in healthy humans. J Appl Physiol 82:760-764, 1997). A positive relationship between physical fitness and formation of NO at rest was found and it was hypothesised that this positive relationship helps to explain the beneficial effects of physical exercise on cardiovascular health. In Jungerstens study nitrate was used solely as a marker of NO production and the authors state several times that nitrate is a stable and inert end product of NO and that it is biologically inactive.

[0011] The present inventors set out to test if administration of dietary nitrate would lead to increased systemic storage pools of nitrite and if this dietary strategy would have an impact on various physiological and biochemical parameters during exercise.

[0012] Crawford et al. (Effect of nitrate on determinants of myocardial oxygen consumption during exercise, International Journal of Cardiology, Vol. 1, No. 3-4, 1 January 1982, pages 307-314) investigated myocardial oxygen demand following the administration of isosorbide dinitrate, an organic nitrate.

[0013] WO 2006/110601 aims inter alia at reducing mortality associated with heart failure; and has investigated the effects of isosorbide dinitrate and/or isosorbide mononitrate. Like the above Crawford et al. article, also this reference is focused on the heart, and while using terms like "improving exercise tolerance" and "improving oxygen consumption" it is clear that increased oxygen consumption is desired. WO 2006/110601 defines improved oxygen consumption as follows: " An increase in a patient's oxygen consumption typically results in the patient's increased exercise tolerance and would imply that the patient would have an improved quality of life".

[0014] KR 2002 0057695 concerns the production of health food based multivitamins using vegetables.

[0015] Cohn et al. (A comparison of enalapril with hydralazine isosorbide dinitrate in the treatment of chronic congestive heart failure, New England Journal of Medicine, Vol. 325, No. 5, 1 january 1991, pages 303-310) concerns a comparison between enelapril and isosorbide dinitrate in the treatment of chronic congestive heart failure, as evident from the title. Using these orgnic nitrates, Cohn et al. observed a vasodilatory effect and increased oxygen consumption.

[0016] US 2004/204371 and US 4,868,179 disclose a comparison between enelapril and isosorbide dinitrate, an organic nitrate, and teach "improving oxygen consumption" which is manifested as increased oxygen consumption These documents are also concerned with isosorbide dinitrate, an organic nitrate. It is also noteworthy that in US 2004/204371 and US 4,868,179 isosorbide dinitrate is combined with hydralazine and not tested alone which makes comparison with inorganic nitrate even less relevant.

[0017] Peri et al. studied the effects of apple extracts on the NO release by human saliva at pH 2 (Peri et al., Apples increase nitric oxide production by human saliva at the acidic pH of the stomach: A new biological function for polyphenols with a catechol group?, Free Radical Biology and Medicine, Vol. 39, No. 5, 1 September 2005, pages 668-681.

[0018] Deng et al. investigated whether ethyl nitrite could be detected in vitro from the reaction of ethanol

with peroxynitrite, as well as after administration of ethanol to mice. (Deng et al., Formation of ethyl nitrite in vivo after ethanol administration, Alcohol, Vol. 34, No. 2-3, 1 October 2004, pages 217-223).

[0019] In two scientific articles, Shibata et al. investigated the oxygen consumption by arteriolar walls in rat skeletal muscle (M Shibata et al., Vascular wall energetics in arterioles during nitric oxide-dependent and - independent vasodilation, Journal of Applied Physiology, 100:1793-1798, 2006) and M. Shibata et al., Nitric oxide modulates oxygen consumption by arteriolar walls in rat skeletal muscle, Am J Physiol Heart Circ Physiol, 289, H2673-H2679, 2005).

Summary of the invention

[0020] The inventors surprisingly found that the performance of a mammal manifested as a reduced oxygen uptake (VO_2) during exercise was enhanced by administering to said mammal a non-toxic amount of nitrate and/or nitrite. Based on this finding, the inventors make available a method for non-therapeutically enhancing the performance of a mammal,. As well as the use of inorganic nitrate or nirite or a combination thereof as defined in the appended claims. The following description is subject to this limitation.

Short description of the figures

[0021] The invention will be described in closer detail in the following description, examples and non-limiting claims, with reference to the attached drawings in which:

Figure 1 shows a graph illustrating numerous ways in which the combination of nitrate and polyphenols synergistically acts to increase the bioavailability of nitric oxide and at the same time to reduce the formation of harmful compounds such as oxygen radicals and nitrosamines. For a detailed explanation see text below.

Figure 2 illustrates the effects of a dietary supplementation with sodium nitrate or sodium chloride (placebo) on plasma concentrations of nitrite measured at rest and immediately after exercise in 9 healthy male volunteers.

Figure 3 shows oxygen consumption (VO_2) and heart rate (HR) measured at 6 different work rates after a 3-day dietary supplementation with sodium nitrate (0.1 mmol/kg/day, NIT) or an equal amount of sodium chloride (CON). The study had a randomized double-blind cross-over design with a washout period of at least 10 days between the tests. * $p<0.05$, ** $p<0.01$.

Figure 4 shows oxygen consumption at 80% of VO_{2Peak} in 9 healthy male volunteers. Measurements were made after a 3-day dietary supplementation with nitrate (0.1 mmol/kg/day) or an equal amount of sodium chloride (placebo). The difference between nitrate and placebo periods was significant ($p<0.01$).

Figure 5 shows plasma lactate concentration measured at 6 different work rates after dietary supplementation with sodium nitrate (0.1 mmol/kg/day for 3 days, filled bars) or an equal amount of sodium chloride (placebo, empty bars).

Detailed description

[0022] Before the present invention is described, it is to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

[0023] It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0024] Also, the term "about" is used to indicate a deviation of +/- 2 % of the given value, preferably +/- 5 %, and most preferably +/- 10 % of the numeric values, where applicable.

[0025] The term "edible" in this context means non-toxic and possible to ingest, however not limited to particular modes of ingesting, such as drinking, chewing, applying to the oral cavity in various forms, such as, for example a spray or aerosol.

[0026] The term "functional food" relates to any fresh or processed food claimed to have a health-promoting and/or disease-preventing property beyond the basic nutritional function of supplying nutrients. Functional foods are sometimes called nutraceuticals. The general category includes processed food made from functional food ingredients, or fortified with health-promoting additives, like "vitamin-enriched" products, and also, fresh foods (e g vegetables) that have specific claims attached. Fermented foods with live cultures are often also considered to be functional foods with probiotic benefits.

[0027] The present inventors showed that dietary supplementation with inorganic nitrate results in a reduced VO_2 during physical exercise and a significant increase in muscular efficiency. These effects occurred without any increase in plasma lactate.

[0028] Many vegetables and fruits are rich in polyphenols. Polyphenols are a group of chemical substances found in plants, characterized by the presence of more than one phenol group per molecule. Polyphenols are generally further subdivided into hydrolyzable tannins, which are gallic acid esters of glucose and other sugars; and phenylpropanoids, such as lignins, flavonoids, and condensed tannins. Thus, in one embodiment of the present invention the nitrate and/or nitrite comprising composition is mixed with a compound that contains high levels of polyphenols. The ratio nitrate comprising composition:polyphenol-rich compound should be chosen to obtain enough supply of nitrate. The nitrate and/or nitrite

comprising composition should therefore be at least about 10%, preferably at least about 20 %, more preferably at least about 30%, even more preferably at least about 40 % and most preferably at least about 50% or even more. It is contemplated that this combined product will have synergistic health promoting effects via potentiation of NO bioavailability. Polyphenols will enhance NO generation by several separate mechanisms highlighted in Figure 1. First, such agents can directly stimulate endogenous NO formation from NO synthase enzymes (1 in figure 1). Second, it is contemplated that these compounds will enhance the reduction of nitrite to bioactive NO due to the presence of reductive -OH groups on the phenol ring (2 in figure 1). Third, by acting as scavengers of free radicals such as superoxide, they prevent these radicals from interacting with (and destroying) NO and thereby NO becomes more long-lived (3 in figure1). In addition to this, nitrite or its reaction products can interact with the polyphenol itself and modify it chemically via nitration or nitrosation reactions (4a in figure 1). The resulting compound can act as a long-lived NO donor (4b in figure 1). An additional effect is that the presence of polyphenols will divert the chemical reactions away from formation of potentially carcinogenic nitrosamines (5 in figure 1). Nitrates reaction product nitrite can react with amines to form nitrosamines but polyphenols will inhibit this reaction by a dual mechanism. First they help to rapidly reduce HNO_2 directly to NO thereby minimizing the formation of nitrosating species (N_2O_3, HNO_2). Second, they can directly compete for nitrosation with the amines by being nitrosated themselves.

[0029] The present inventors consider presenting the composition to the market in the form of a sport drink, an energy drink, a sport bar, or an energy bar.

[0030] The energy bar may take on a variety of forms. For convenience, it is preferred that the energy food product be shaped like a box, square, cylinder, string, pie, sphere, triangle, or other format suitable for packaging, transportation, handling and eating.

[0031] According to another embodiment, the composition is presented to the market as a functional food product.

[0032] Products comprising the inorganic nitrate, nitrite or a combination thereof can easily be manufactured by persons skilled in the food, sweets and beverage industry or the pharmaceutical industry, and existing compositions supplemented with nitrate, nitrite and other combinations described herein in amounts according to this invention.

[0033] For example an energy bar according to the present invention may include, in addition to nitrate and optionally nitrite, also a variety of other components such as, for example, nuts, crisps, fruit pieces, chocolate, seeds, and the like. Preferred nuts are almonds, peanuts, hazelnuts, cashews, walnuts, pecans, brazil nuts, and the like. Crisp components include rice crisps, corn crisps, oats, wheat flakes, and the like. The chocolate can be any type of chocolate or chocolate like edible component in various forms, such as, for example, chocolate chips, chunks, flakes and the like. Non-limiting examples of seeds include sesame, sun flower, poppy, caraway, fennel and the like.

[0034] Additionally, traditional food ingredients such as flavours and the like may be included. For example, additional ingredients may include natural and artificial flavours, sweeteners, salt, flavour enhancers, colour additives, emulsifiers, stabilizers, fats, preservatives, and the like.

[0035] The present invention also makes available a second non-medical use of inorganic nitrate and/or nirite, i.e. for the manufacture of a composition for enhancing the performance of a mammal wherein the effect of enhancing performance is manifested as reduced VO_2 during physical exercise

[0036] Preferably the effect of enhancing performance is manifested as both a reduced VO_2 during physical exercise and a significant increase in muscular efficiency.

[0037] According to an embodiment of the above use, nitrate and nitrite is used in a dose ratio interval of about 5:1 to about 100:1 (nitrate:nitrite), such as 5:1, 10:1, 30:1, 50:1, 70:1 and 100:1. Preferably the dose ratio is about 10:1.

[0038] According to a particular embodiment, said inorganic nitrite is used alone, without the presence of nitrate.

[0039] According to an embodiment of the invention, the source of inorganic nitrate/nitrite is chosen among a concentrate or an extract of nitrate containing vegetables, or an inorganic nitrate or nitrite salt. Examples of nitrate and nitrite salts include but are not limited to sodium, potassium, calcium, zinc, arginine, and ammonium. Examples of vegetables rich in nitrates are green leafy vegetables, spinach, beetroot, fennel, lettuce, cabbage and the like. Juices, pastes, concentrates etc of such vegetables are contemplated as suitable sources of nitrate.

[0040] Combinations of nitrate and nitrite salts can also be used. According to one embodiment, nitrate and nitrite are given orally in a dose ratio interval of about 5:1 to about 100:1 (nitrate:nitrite), such as 5:1, 10:1, 30:1, 50:1, 70:1 and 100:1. Preferably the dose ratio is about 10:1. This will provide the acute effects of the nitrite as soon as it is absorbed, and then provide a sustained effect of the nitrate following its bioconversion into nitrite.

[0041] In yet another embodiment inorganic nitrate and/or nitrite is used together with polyphenols. The characteristics and health promoting effects of polyphenols are described above. The ratio inorganic nitrate and/or nitrite composition:polyphenol-rich compound should be chosen as to obtain enough supply of nitrate/nitrite. The nitrate/nitrite comprising composition should therefore be at least about 10%, preferably at least about 20 %, more preferably at least about 30%, even more preferably at least about 40 % and most preferably at least about 50% or even more.

[0042] Examples of fruit and fruit juices rich in polyphe-

nols that can be used are, but not limited to, apple, pear, grapes, lemon, orange, lime, peach, pomegranate, grapefruit, kiwi, ginger and pineapple. Berries and juice from berries are also usable such as, but not limited to, blackberries, black raspberries, blueberries, cranberries, red raspberries, cherries, bog wortleberry, lingonberries, black elderberry, black chokeberry, black currant, blueberry, cloudberries and strawberries. Other natural sources of polyphenols include, but are not limited to, vegetables such as carrots, chili, rhubarb, onions. In addition, cacao products (rich in flavanols), green or black tea, nuts, Yerba mate and coffee are all rich in polyphenols. In one preferred embodiment beetroot (such as beetroot juice) is used as a source of inorganic nitrate together with one or several polyphenol-rich products. The ratio beetroot juice: polyphenol-rich compound should be chosen to obtain enough supply of nitrate and therefore the beetroot juice part should be at least about 10%, preferably at least about 20 %, more preferably at least about 30%, even more preferably at least about 40 % and most preferably at least about 50%.

[0043] In another embodiment of the present invention non-pathogenic bacteria are used in combination with inorganic nitrate and/or nitrite or the composition containing a blend of inorganic nitrate and/or nitrite and polyphenol-rich compounds. The combination with bacteria can be in the form of a drink such as a juice, a yoghurt, a milk based drink or any other fermented food product. The combination with bacteria can also be included in different types of functional food products. Suitable bacteria are the so called probiotic bacteria, included but not limited to Lactobacilli (for example *L. acidophilus, L. delbrueckii, L. helveticus, L. salivarius, L. casei, L. curvatus, L. plantarum, L. sakei, L. brevis, L. buchneri, L. fermentum, L. reuteri)* and bifidobacteria species, for example, but not limited to, *B.breve, B. bifidum, B. lactis)* and probiotic yeasts such a Saccharomyces boulardii. Suitable non-pathogenic bacteria are for example, but not limited to, *Staphylococcus* species, *Actinomyces* species and *Rothia* species. These microorganisms may also be used in "dry form".

[0044] Contamination of a nitrate-containing food or drink with bacteria may result in a large accumulation of nitrite, due to nitrate reducing bacterial enzymes. Ingestion of high levels of nitrite may cause potentially serious methemoglobinemia. In one embodiment a nitrate-rich composition is used together with a compound that inhibits bacterial growth. Such compound should be chosen so as not to affect the taste of the product negatively. Ideally, it should enhance the taste and at the same time increase the bioactivity of the product. One option is to acidify the juice so that final pH is below about 5, and most preferably between about pH 2-4. This will inhibit and/or abolish bacterial growth. Suitable acidifying agents can be any agent that reduces pH and include artificial compounds as well as natural juices from e.g, but not limited to, lemon or lime, ascorbic acid, acetic acid or vinegar (from apple, grapes or other fruits). It is contemplated that with the use of natural products a dual effect is achieved. Besides having an antibacterial effect, they are rich in polyphenols, which enhance the generation of bioactive NO from nitrate/nitrite in the vegetable drink. In one particular embodiment a nitrate-rich vegetable juice (e.g. beetroot juice) is used together with a compound that inhibits bacterial growth.

[0045] In another embodiment of the present invention a low concentration of ethanol is used together with inorganic nitrate and/or nitrite. For example, if the inventive composition is in the form of a liquid, the ethanol content should be below about 5 % (v/v), more preferably below about 2% (v/v), and most preferable between about 0.5-1 % (v/v).

[0046] In yet another embodiment of the present invention a nitrate and/or nitrite salt (for example potassium nitrate or ammonium nitrate) or a natural nitrate source including a dried vegetable powder, is used in combination with liquorice, for example salty liquorice (ammonium chloride). The use of polyphenols to this combination is also preferred. In particular a salt such as potassium nitrate, sodium nitrate or ammonium nitrate may be used to replace in part or in whole the salt content (such as sodium chloride or ammonium chloride) of the liquorice product.

[0047] The composition of the present invention may be manufactured into liquids, pastes, bars, cakes, powders, granulates, effervescent tablets, tablets, capsules, lozenges, chewing gum, fast melting tablets or wafers, sublingual tablets, a spray or the like, using conventional methods practiced in the food, sweets and pharmaceutical industry.

[0048] Further the composition may also be manufactured in the form of or as a part of a food product, such as a liquid, a paste a bar, a cake a powder or a granulate. It may for example be in the form of a fermented food product, a functional food product, or a sport drink or the like as mentioned above. Another composition is a nicotine-free smokell tobacco and/or wet snuff. Such products an be manufactured using conventional methods practised in the food and beverage industry, or in pharmaceutical industry.

[0049] In one embodiment of the present invention an inorganic nitrate and/or nitrite is used in combination with a cacao product such as dark chocolate that is rich in flavanols. One preferred nitrate-rich compound in this embodiment is rhubarb.

[0050] In yet another embodiment inorganic nitrate and/or nitrite is used in combination with a compound that inhibits bacterial growth. One option is to acidify the juice so that final pH is below about 5, and most preferably between about pH 2-4. This will inhibit and/or abolish bacterial growth. Suitable acidifying agents can be any agent that reduces pH and include artificial compounds as well as natural juices from e.g, but not limited to, lemon or lime, ascorbic acid, acetic acid or vinegar (from apple, grapes or other fruits). It is contemplated that with the use of natural products a dual effect is achieved. In one

particular embodiment a nitrate-rich vegetable juice (e.g. beetroot juice) is used in combination with a compound that inhibits bacterial growth.

[0051] The inventors also make available a method for non-therapeutically enhancing the performance of a mammal, wherein inorganic nitrate and/or nitrite is administered to said mammal. Said mammal is chosen among a human, a horse, or a dog, preferably a human. Preferably the dose of nitrate is about 1 - 1000 umol sodium nitrate/kg bodyweight/day. Correspondingly, the dose of nitrite is preferably about 0.1-100 umol/kg bodyweight/day.

[0052] More specifically, for the use of a nitrate salt perorally, a dose of 0.01-10 mmol/kg/24h, even more preferably 0.1 - 1 mmol/kg/24h. Correspondingly, the dose of nitrite is 0,001-1 mmol/kg/24 h and more preferably about 0.0001-0,1 mmol/kg/24 h.

[0053] According to an embodiment of the invention, nitrate and nitrite is administered in a dose ratio interval of about 5:1 to about 100:1 (nitrate:nitrite), such as 5:1, 10:1, 30:1, 50:1, 70:1 and 100:1. Preferably the dose ratio is about 10:1.

[0054] According to one particular embodiment, only nitrite is administered.

[0055] In yet another embodiment of the inventive method inorganic nitrate and/or nitrite is administered together with polyphenols. The characteristics and health promoting effects of polyphenols are described above. The ratio inorganic nitrate and/or nitrite composition:polyphenol-rich compound should be chosen to obtain enough supply of nitrate. The nitrate/nitrite comprising composition should therefore be at least about 10%, preferably at least about 20 %, more preferably at least about 30%, even more preferably at least about 40 % and most preferably at least about 50% or even more. Examples of products rich in polyphenols that can be administered are outlined above.

[0056] In another embodiment of the present invention non-pathogenic bacteria are administered in combination with inorganic nitrate and/or nitrite or the composition containing a blend of inorganic nitrate and/or nitrite and polyphenol-rich compounds. Non-limiting examples of suitable bacteria for administration are outlined above.

[0057] In another embodiment of the inventive method inorganic nitrate and/or nitrite is administered together with a low concentration of ethanol. For example, if the inventive inorganic nitrate and/or nitrite composition is in the form of a liquid, the ethanol content should be below about 5 % (v/v), more preferably below about 2% (v/v), and most preferable between about 0.5-1% (v/v).

[0058] In yet another embodiment of the present invention a nitrat or nitrite salt (for example potassium nitrate or ammonium nitrate) or a natural nitrate source including a dried vegetable powder, is administered in combination with liquorice for example salty liquorice (ammonium chloride). The administration of polyphenols to this combination is also preferred. In particular a salt such as potassium nitrate, sodium nitrate or ammonium nitrate may be used to replace in part or in whole the salt content (such as sodium chloride or ammonium chloride) of the liquorice product.

[0059] In one embodiment of the present invention an inorganic nitrate and/or nitrite is administered in combination with a cacao product such as dark chocolate that is rich in flavanols. One preferred nitrate-rich compound in this embodiment is rhubarb.

[0060] There is reason to believe that the observed effects of nitrate on physical performance involve initial reduction of nitrate to nitrite. Nitrate itself is believed to be biologically inert and cannot be metabolised by mammalian cells. However, after ingestion nitrate re-enters into the mouth via the salivary glands and is effectively reduced by commensal bacteria thereby forming nitrite. In contrast to nitrate the nitrite ion has recently been shown to possess a wide range of bioactivities.

[0061] The inventors noted an increase in plasma nitrite after the nitrate treatment period thereby confirming in vivo reduction of nitrate as described previously (Lundberg & Govoni 2004, LARSEN, F J, et al. Effects of dietary nitrate on blood pressure in healthy volunteers. N Engl J Med. 2006, vol.255, no.26, p.2792-3). Another finding in support of nitrite being bioactive was its effective consumption during exercise in contrast to the unchanged levels of plasma nitrate. Ultimately the bioactivity of nitrite is likely related to its further reduction to NO and possibly other closely related nitrogen intermediates. In addition, it has been recently suggested that nitrite itself may directly affect cellular signalling pathways (BRYAN, N S, et al. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. Nat Chem Biol. 2006, vol.1, no.5, p.290-7). Although probably unlikely, at this early stage, effects of the nitrate ion itself cannot be excluded. There are several principle ways by which biological effects of nitrogen oxides may be propagated including alteration of protein function by nitrosylation, nitration or direct binding to protein hememoieties as in the prototypic activation of guanylyl cyclase by NO.

[0062] Earlier studies using NOS inhibitors to block endogenous NO production give some indications. NOS-inhibition has been shown to increase submaximal VO_2 in dogs during exercise, independently of the reduction in blood flow (SHEN, W, et al. Nitric oxide. An important signaling mechanism between vascular endothelium and parenchymal cells in the regulation of oxygen consumption. Circulation. 15 Dec 1995, vol.92, no.12, p.3505-12., ISHIBASHI, Y, et al. ATP-sensitive K+-channels, adenosine and NO-mediated mechanisms account for coronary vasodilation during exercise. Circulation Res. 1998, no.82, p.346-359.; Shen *etal.* 1999, supra). The increase in VO_2 during NOS-blockade has been explained by the fact that NO affects tissue respiration by reversible inhibition of the respiratory enzyme cytochrome c oxidase (Carr & Ferguson 1990, supra; Bolanos *et al.* 1994, supra; Brown & Cooper 1994, Cleeter *et al.* 1994, Schweizer & Richter 1994). Others have related the increased

VO_2 during NOS-blockade to an inhibiting effect of NO on proton leakage over the inner mitochondrial membrane (BOHUSLAVS'KYI, A, et al. Effect of nitric oxide on the efficiency of oxygen consumption by the working skeletal muscle in fatigue. FiziolZh. 2005, vol.51, no.1, p.33-42; NAVET, R, et al. Proton leak induced by reactive oxygen species produced during in vitro anoxia/reoxygenation in rat skeletal muscle mitochondria. J Bioenerg Biomembr. 2006, vol.38, no.1, p.23-32; WANG, G, et al. Nitric oxide donors protect murine myocardium against infarction via modulation of mitochondrial permeability transition. Am J Physiol Heart Circ Physiol. 2005, vol.288, no.3, p.1290-5). If the effects of nitrate were solely due to inhibition of cytochrome c oxidase one would expect an increase in anaerobic metabolism during physical exercise and a larger accumulation of lactate. However, judging from the results this was not the case, as the plasma lactate concentration was near identical after nitrate supplementation compared to placebo. The inventors consider this to be very surprising.

[0063] The studies using NOS inhibitors cited above all imply that endogenous NO is involved in regulation of oxygen consumption but there have been few attempts to study the effect of exogenous NO delivery. Studies with NO-donors such as nitroprusside and nitroglycerine have shown somewhat diverging results, with decreases in VO_2 in some cases (RECCHIA, F A, et al. Nitric oxide controls cardiac substrate utilization in the conscious dog. Cardiovasc Res. 1999, no.44, p.325-32; LOKE, K E, et al. Nitric oxide modulates mitochondrial respiration in failing human heart. Circulation. 21 Sept 1999, vol. 100, no.12, p.1291-7), no effect in one study (NUNEZ, C, et al. Discrepancies between nitroglycerin and NO-releasing drugs on mitochondrial oxygen consumption, vasoactivity, and the release of NO. Circ Res. 11 Nov 2005, vol.97, no.10, p.1063-9) and increases in other settings (DE BACKER, D, et al. Effects of dobutamine on the relationship between oxygen consumption and delivery in healthy volunteers: comparison with sodium nitroprusside. Clin Sci (Lond). 1996, vol.90, no.2, p.105-11).

[0064] Several of the proposed mechanisms for nitrite reduction to NO described above could theoretically come into play during physical exercise. Thus, all these pathways are greatly enhanced during hypoxia and when pH decreases such as in a working muscle. Shiva and colleagues very recently demonstrated deoxymyoglobin-dependent nitrite reduction to NO in rat heart homogenates with a concomitant inhibition of mitochondrial respiration (Shiva et al 2007, supra). Another possible pathway includes NO formation by the mitochondria themselves (KOZLOV, A V, et al. Nitrite reductase activity is a novel function of mammalian mitochondria. FEBS Lett. 2 Jul 1999, vol.454, no. 1-2, p.127-30) or even simple acidic reduction of nitrite in the working muscle (Zweier *et al.* 1995, *supra,* MODIN, A, et al. Nitrite-derived nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. Acta Physiol Scand. 2001, vol.171, no.1, p.9-16). Cosby and colleagues described NO formation and vasodilation from the reaction of circulating nitrite ions with deoxyhemoglobin in blood (COSBY, K, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med. 2003, vol.9, no.12, p.1498-505). While this latter pathway, and possibly also tissue nitrite reduction, very well might explain the recently described nitrate-induced reduction in resting blood pressure (Larsen *et al.* 2006), it is still not obvious how this NO also would decrease oxygen consumption in the working muscle. Thus, an effective inhibition of mitochondrial respiration e.g. by deoxymyoglobin-derived NO, would again be expected to result in accumulation of plasma lactate which was not the case.

[0065] The efficiency of the muscles to produce work has been related to the percentage of type I muscle fibres (COYLE, E F, et al. Cycling efficiency is related to the percentage of type I muscle fibers. Med Sci Sports Exerc. 1992, vol.24, no.7, p.782-8) and uncoupling protein-3 (UCP3) content of muscle fibres (MOGENSEN, M, et al. Cycling efficiency in humans is related to low UCP3 content and to type I fibers but not to mithochondrial efficiency. J Physiol. 2006, vol.571, no.3, p.669-681). Other factors that might contribute to the efficiency of movement are anatomical, biochemical and biomechanical features (WILLIAMS, K R. The relationship between mechanical and physiological energy estimates. Med Sci Sports Exerc. 1985, no.17, p.317-25). Thus, simply measuring differences in VO_2 at different work rates is not an optimal estimate of muscular efficiency because the energy output for a certain VO_2 is dependent upon substrate utilization. Gross efficiency (GE) calculations include possible changes in respiratory exchange ratio and thereby take substrate utilization into account. The improved GE after nitrate supplementation indicates better efficiency, but even so, it cannot be exclude that this improved efficiency originates from reduced baseline energy expenditure (EE). The Delta efficiency (DE) calculations are not dependent on the baseline EE and are also based on all work rates taken together instead of a single work rate at a time as in the GE-calculations. It is therefore plausible to expect DE to be the most valid estimate of muscular efficiency in this case. Indeed, even DE was significantly improved after nitrate supplementation. It is unlikely that the improved efficiency by nitrate comes from mechanical factors. The subjects of this study were all cyclists with many years of experience of training and competing. It is improbable that a few visits to the laboratory would change their efficiency during cycling to any noteworthy extent. Especially since the subjects used the same cycling shoes, clip-on pedals and the same seat position as they where used to during training makes this even more unlikely. More important, the randomization procedure used in this study rules out any such differences. Marcheal and Gailly (MARCHEAL , G, et al. Effects of nitric oxide on the contraction of skeletal muscle. CellMolLife Sci. 1999, no.55, p.1088-1102) demonstrated a faster relaxing velocity of muscle fibres in *in situ* experiments during administration of an NO-donor,

thereby implicating a neuromuscular modulatory effect of NO. It remains to be proven if this can improve the muscular efficiency during cycling.

[0066] The finding that the oxygen pulse at a given work rate decreases by nitrate supplementation is a direct effect of the lower oxygen demand at that work rate. However, there is no difference in oxygen pulse at a given absolute oxygen uptake. The lack of effect of nitrate on VE/VO_2 or oxygen pulse indicates that the improved efficiency originates from muscular or mitochondrial adaptations rather than from central adaptations in the heart or the lungs.

[0067] In summary, the present findings demonstrate a lower oxygen cost during submaximal work after dietary supplementation with nitrate, in amounts achievable through the intake of a non-toxic amount of nitrite. This occurred without an accompanying increase in plasma lactate, indicating that the energy production had become more efficient. The mechanism of action and main targets need to be clarified but the process likely involves *in vivo* reduction of nitrate into bioactive nitrogen oxides including nitrite and NO.

Examples

Methods

Subjects

[0068] Nine healthy, well-trained (VO_{2peak} 55 +/-3.7 ml x kg^{-1} x min^{-1}), males (28 +/-6 years) volunteered for the study. All subjects were trained cyclists or triathletes and well accustomed to the testing procedure. The inventors in this study chose to use well-trained subjects to avoid training effects from the tests such as enhanced VO_{2peak} or better mechanical efficiency during submaximal exercise. The protocol was approved by the regional ethics committee in Stockholm and all subjects gave their written consent prior to participation.

Dietary supplementation with nitrate

[0069] The aim with the present study was to investigate the effects of two distinct dietary patterns, one with higher, and one with lower than normal nitrate intake. The study had a double-blind placebo-controlled cross-over design. During two three-day periods, separated by a washout interval of ten days, the subjects were instructed to avoid all foods with moderate or high nitrate content (all vegetables, all cured meats, strawberries, grapes, and tea). In addition, they were told to restrain from alcohol and tobacco products. Otherwise they were free to eat any food they liked during the three days of restricted diet. The subjects were randomized to start with either ingestion of 0.1 mmol sodium nitrate/kg bodyweight/day dissolved in water or an equal amount of sodium chloride (placebo). The daily dose was divided and ingested three times daily. The different solutions could not be distinguished by taste or appearance. The daily nitrate dose corresponded to the amount normally found in 150-250 gram of a nitrate-rich vegetable such as spinach, lettuce or beetroot (Lundberg et al, 2004, *supra*). The last dose of nitrate or placebo was ingested in the morning on the day of measurement (see the main tests below). The order between the nitrate supplementation period (NIT) and the placebo period (CON) was balanced. During the washout period the subjects did not adhere to any specific dietary regime.

Experimental protocol

[0070] Measurements were carried out on an electrically braked cycle ergometer (Monark 839E, Varberg, Sweden) that was modified with a racing saddle and the pedal system the subjects were familiar with from training. The bicycle ergometer was computer-controlled, permitting a constant work rate regardless of the cadence the subject chose to pedal with. The pedalling cadence was individually chosen in the range of 70-90 rpm but kept constant during the test to minimize differences in work output due to changes in muscle recruitment patterns.

[0071] Pulmonary ventilation (V_E), oxygen uptake (VO_2), CO_2 output (VCO_2) and respiratory exchange ratio (RER) were measured at 10 second intervals by a computerised gas analyser (AMIS 2001, Odense, Denmark) connected to a flow meter which the subjects breathed through via a mouthpiece and a plastic tube. Heart rate (HR) was continuously recorded during the tests with a portable heart rate monitor (Polar S610, Polar, Kempele, Finland). Capillary blood samples (20µl) were collected from the fingertip and were analyzed for lactate ([Hla]) using a Biosen C-Line Sport Analyser (EKF diagnostics, Magdeburg, Germany). Haemoglobin concentration ([Hb]) at rest was determined with capillary blood taken from the fingertip and analyzed with an Hb-measuring device (Hemocue, Ängelholm, Sweden). Hematocrit (Hct) was determined by centrifuging capillary blood at 12000 rpm for three minutes.

Pre-tests:

[0072] Each subject attended the laboratory twice within a two week period before the first main tests. The first pre-test was carried out to familiarize the subject with the bicycle ergometer and the testing procedure. The subjects did a preliminary test at five submaximal levels with every level lasting for five minutes. There was no rest between the different submaximal levels. VO_2 was continuously measured with the AMIS 2001. At the end of each submaximal level capillary blood was taken from the fingertip and later analysed for [Hla]. At every work rate the subjects rated their perceived exertion on the Borg's RPE-scale (BORG, G. Perceived exertion as an indicator of somatic stress. Scand J Rehabil Med. 1970, vol.2, no.2, p.92-8), both central and muscular exertion

were rated. After eight minutes of recovery, the subject was instructed to cycle for as long as possible at a work rate corresponding to his calculated maximal oxygen uptake (ÅSTRAND, P O, et al. Textbook in work physiology. New York: McGraw-Hill Book Company, 1970. p.619). During this test the subjects actual VO_{2peak} was measured and if the subject was able to cycle for longer than seven minutes extra power of 20-30 watts was added every minute until exhaustion. One and three minutes after the maximal test capillary blood were sampled from the fingertip for analysis of [Hla].

[0073] Before the second pre-test, the submaximal levels were adjusted so that they corresponded to 45, 60, 70, 80 and 85% of VO_{2peak}. The maximal work rate was also adjusted, if necessary, so that the time to exhaustion was kept between four and seven minutes.

The main tests:

[0074] The subjects refrained from heavy exercise three days prior to the main tests and avoided all exercise the day before the tests. They were also told to eat their last light meal at least 3 hours before the start of the tests. When the subjects came to the laboratory they received their last dose of either placebo or nitrate and were allowed to rest in the supine position for 60 minutes before the test commenced.

[0075] All subjects used a standardised warm up procedure of five min of cycling at 100 watts followed by five minutes of rest. The submaximal and maximal tests were performed in the same way as the second pre-test with five submaximal work rates lasting five minutes each, without rest between the different levels. Identical work rates were used during the two main tests. Venous blood (9 ml) was drawn at rest 45 minutes after the last nitrate/placebo-dose was ingested and again immediately after the VO_{2peak}-test. The blood was placed in an ice bath and centrifuged within five minutes at 1300 rpm and 4°C. The plasma was separated and kept at -80°C until it was analysed for its nitrate and nitrite concentrations by a chemiluminescence assay as described previously (Lundberg 2004, supra).

Statistics and calculations:

[0076] Results are expressed as means +/- standard deviation (mean +/-SD). Paired t-tests were used to evaluate the difference between the nitrate and the placebo trials. The significance level was set as p=<0.05.

[0077] Gross efficiency (GE) was defined as the work rate divided by the actual energy expenditure (EE). The EE was in turn calculated with the Brouwer equation (BROUWER, E. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. Acta Physiol Pharmacol Neerl. 1957, no.6, p.795-802). Delta efficiency (DE) was defined as the increase in work rate divided by the increase in EE (GAESSER, G A, et al. Muscular efficiency during steady-rate exrcise: effect of speed and work rate. J Appl Physiol. 1975, no.38, p.1132-1139). The DE was based on the four lowest work rates and was analyzed with linear regression. The oxygen pulse is defined as VO_2/HR.

Results:

Blood pressure at rest:

[0078] Average resting systolic blood pressure was lower after nitrate supplementation (112 +/- 8 mmHg) compared to placebo (120 +/- 5.9, p<0.01). The diastolic blood pressure was also lower after nitrate (68 +/-5.5 mmHg) compared to placebo (74 +/- 6.8 mmHg, p<0.01). Parts of these findings have been published as a separate communication (Larsen *et al.* 2006).

Blood values:

[0079] No change was observed in [Hb] at rest (NIT 152 +/-11, CON 153 +/-11 g x l^{-1}, p=0.87) or immediately after the VO_{2peak}-test (NIT 163 +/- 13, CON 161 +/-13 g x l^{-1}, p=0.27). Nor were there any change in the hematocrit value at rest (NIT 42 +/-4, CON 43 +/-3%, p=0.19) or after the VO_{2peak}-test (NIT 46 +/-4, CON 47 +/-4%, p=0.6).

[0080] Plasma levels of nitrate at rest were 27 +/- 6.9 μM in CON and 182 +/- 55 in NIT (p=<0.01). Nitrate levels immediately after the maximal work test were 29 +/-6.1 in CON and 175 +/- 61 μM in NIT (p=<0.01). Plasma nitrate did not change during exercise either in NIT or in CON (p=0.8). Nitrite levels at rest were 124 +/- 28 in CON and 226 +/- 87 nM in NIT (p=<0.01). Immediately after the maximal work test the nitrite levels were 111 +/- 29 in CON and 137 +/- 48 in NIT (p=0.17). The decrease in nitrite concentrations during exercise was more pronounced in NIT than in CON (See Fig 2).

Work parameters:

[0081] After nitrate administration VO_2 was significantly lower during the five work rates corresponding to 45-80% VO_{2peak} compared to the placebo period (Fig 3). The most significant difference was seen at 80% of VO_{2peak} (NIT 3.44 +/-0.31 l x min^{-1} vs CON 3.61 +/-0.31 l x min^{-1}, p=0.003, Fig 4). On average VO_2 was 0.15 l x min^{-1} lower in the NIT-trials over the five submaximal work rates. There was no difference in heart rate (HR) between the NIT and CON-trials (see Fig 3). The oxygen pulse tended to decrease from 21.0 +/- 2.0 during CON to 20.3 +/- 1.9 ml x $beat^{-1}$ (p=0.08). No differences were found between NIT and CON in [Hla] (Fig 5), V_E, V_E/VO2 or respiratory exchange ratio (RER) during any of the submaximal work rates. The average gross efficiency improved from 19.7% during CON to 21.1% during NIT (p=0.02). Delta efficiency (DE) increased significantly

from 22.1 +/- 1.6 % during CON compared to 22.9 +/- 1.9 % during NIT, (p=0.04).

[0082] At maximum work rate the VO_2peak values for NIT and CON trials was 4.49 +/- 0.44 and 4.61 +/- 0.28 l x min^{-1} respectively. No significant differences were noted either in V_{Emax} (NIT 182 +/- 21.4 vs CON 186 +/-21.7 l x min^{-1}, p=0.5), HR_{max} (NIT 189.8 +/- 7.0 vs CON 190.3 +/- 7.5 beats x min^{-1}, p=0.94) or maximal work rate (NIT 360.6 +/- 32.8 vs CON 358.9 +/- 32.3 watt, p=0.35). There was no difference between NIT and CON in the rating of perceived exertion (Borg RPE-scale) at any work load (submax or max).

Claims

1. A method for non-therapeutically enhancing the performance of a mammal, said enhanced performance manifested as reduced oxygen consumption (VO_2) during physical exercise, **characterized in that** inorganic nitrate, nitrite or a combination thereof is administered perorally, wherein the nitrate dose is 0.01 - 10 mmol sodium nitrate/kg bodyweight/day, the nitrite dose is 0.001-1 mmol/kg bodyweight/day, and wherein the dose ratio interval when nitrate and nitrite are administered in combination is 5:1 to 100:1 (nitrate:nitrite), wherein said performance is enhanced as compared with the physical performance of said mammal during said physical exercise in the absence of said administration of inorganic nitrate and/or nitrite.

2. The method according to claim 1, wherein said nitrate and/or nitrite is administered together with polyphenols.

3. The use of inorganic nitrate or nitrite or a combination thereof for non-therapeutically enhancing the performance of a mammal, said enhanced performance manifested as reduced oxygen consumption (VO_2) during physical exercise, **characterized in that** inorganic nitrate, nitrite or a combination thereof is administered perorally, wherein the nitrate dose is 0.01 - 10 mmol sodium nitrate/kg bodyweight/day, the nitrite dose is 0.001-1 mmol/kg bodyweight/day, and wherein the dose ratio interval when nitrate and nitrite are administered in combination is 5:1 to 100:1 (nitrate:nitrite), wherein said performance is enhanced as compared with the physical performance of said mammal during said physical exercise in the absence of said administration of inorganic nitrate and/or nitrite.

4. The use according to claim 3 , wherein said composition has the form of a liquid, a paste, a bar, a cake, a powder, a granulate, an effervescent tablet, a tablet, a capsule, a lozenge, a chewing gum, a fast melting tablet or wafer, a sublingual tablet or a spray.

5. The use according to any one of claims 3 - 4, wherein said composition has the form of a sport drink, an energy drink, a sport bar, or an energy bar.

6. The use according to any one of claims 3 - 5, wherein the source of inorganic nitrate is chosen among a concentrate, an extract or a juice of nitrate-containing vegetables or an inorganic nitrate salt.

7. The use according to any one of claims 3 - 6, wherein said inorganic nitrate is used in combination with polyphenols.

8. The use according to any one of claims 3 - 7, wherein said inorganic nitrate is used in combination with live bacteria capable of enhancing nitrate or nitrite reduction.

9. The use according to any one of claims 3 - 8, wherein said inorganic nitrate is used in combination with ethanol in an amount below 5 % (v/v), preferably below 2 % (v/v), more preferably between 0.5 - 1 % (v/v).

Patentansprüche

1. Verfahren zum nichttherapeutischen Verstärken der Leistung eines Säugetiers, wobei sich die verstärkte Leistung als reduzierter Sauerstoffverbrauch (VO_2) bei körperlicher Betätigung äußert, **dadurch gekennzeichnet, dass** anorganisches Nitrat, Nitrit oder eine Kombination davon peroral verabreicht wird, wobei die Nitratdosis 0,01 bis 10 mmol Natriumnitrat/kg Körpergewicht/Tag beträgt, die Nitritdosis 0,001 bis 1 mmol/kg Körpergewicht/Tag beträgt und wobei das Dosisverhältnisintervall, wenn Nitrat und Nitrit in Kombination verabreicht werden, 5:1 bis 100:1 (Nitrat:Nitrit) beträgt, wobei die Leistung im Vergleich zu der körperlichen Leistung des Säugetiers während der körperlichen Betätigung ohne die Verabreichung von anorganischem Nitrat und/oder Nitrit verstärkt wird.

2. Verfahren nach Anspruch 1, wobei das Nitrat und/oder das Nitrit zusammen mit Polyphenolen verabreicht wird/werden.

3. Verwendung von anorganischem Nitrat oder Nitrit oder einer Kombination davon zum nichttherapeutischen Verstärken der Leistung eines Säugetiers, wobei sich die verstärkte Leistung als reduzierter Sauerstoffverbrauch (VO_2) bei körperlicher Betätigung äußert, **dadurch gekennzeichnet, dass** anorganisches Nitrat, Nitrit oder eine Kombination davon peroral verabreicht wird, wobei die Nitratdosis 0,01 bis 10 mmol Natriumnitrat/kg Körpergewicht/Tag beträgt, die Nitritdosis 0,001 bis 1 mmol/kg Körpergewicht/Tag beträgt und wobei das Dosisver-

hältnisintervall, wenn Nitrat und Nitrit in Kombination verabreicht werden, 5:1 bis 100:1 (Nitrat:Nitrit) beträgt, wobei die Leistung im Vergleich zu der körperlichen Leistung des Säugetiers während der körperlichen Betätigung ohne die Verabreichung von anorganischem Nitrat und/oder Nitrit verstärkt wird.

4. Verwendung nach Anspruch 3, wobei die Zusammensetzung die Form einer Flüssigkeit, einer Paste, eines Riegels, eines Kuchens, eines Pulvers, eines Granulats, einer Brausetablette, einer Tablette, einer Kapsel, einer Lutschtablette, eines Kaugummis, einer schnell schmelzenden Tablette oder eines Blättchens, einer Sublingualtablette oder eines Sprays aufweist.

5. Verwendung nach einem der Ansprüche 3 bis 4, wobei die Zusammensetzung die Form eines Sportgetränks, eines Energiegetränks, eines Sportriegels oder eines Energieriegels aufweist.

6. Verwendung nach einem der Ansprüche 3 bis 5, wobei die Quelle von anorganischem Nitrat aus einem Konzentrat, einem Extrakt oder einem Saft nitrathaltige Gemüse oder einem anorganischen Nitratsalz ausgewählt ist.

7. Verwendung nach einem der Ansprüche 3 bis 6, wobei das anorganische Nitrat in Kombination mit Polyphenolen verwendet wird.

8. Verwendung nach einem der Ansprüche 3 bis 7, wobei das anorganische Nitrat in Kombination mit lebenden Bakterien verwendet wird, die zum Verstärken der Nitrat- oder Nitritreduktion in der Lage sind.

9. Verwendung nach einem der Ansprüche 3 bis 8, wobei das anorganische Nitrat in Kombination mit Ethanol in einer Menge von unter 5 % (Vol./Vol.), vorzugsweise von unter 2 % (Vol./Vol.), mehr bevorzugt von 0,5 bis 1 % (Vol./Vol.) verwendet wird.

Revendications

1. Procédé d'amélioration non thérapeutique de la performance d'un mammifère, ladite performance améliorée se manifestant comme une consommation d'oxygène réduite (VO_2) durant un exercice physique, **caractérisé en ce que** du nitrate inorganique, du nitrite inorganique ou une combinaison de ceux-ci est administré(e) par voie orale, dans lequel la dose de nitrate est de 0,01 à 10 mmol de nitrate de sodium/kg de poids corporel/jour, la dose de nitrite est de 0,001 à 1 mmol/kg de poids corporel/jour, et dans lequel l'intervalle de rapport de dose lorsque le nitrate et le nitrite sont administrés en combinaison est de 5/1 à 100/1 (nitrate/nitrite), dans lequel ladite performance est améliorée relativement à la performance physique dudit mammifère durant ledit exercice physique en l'absence de ladite administration de nitrate et/ou de nitrite inorganiques.

2. Procédé selon la revendication 1, dans lequel ledit nitrate et/ou ledit nitrite sont administrés conjointement à des polyphénols.

3. Utilisation de nitrate ou nitrite inorganiques ou d'une combinaison de ceux-ci pour l'amélioration non thérapeutique de la performance d'un mammifère, ladite performance améliorée se manifestant comme une consommation d'oxygène réduite (VO_2) durant un exercice physique, **caractérisée en ce que** du nitrate inorganique, du nitrite inorganique ou une combinaison de ceux-ci est administré(e) par voie orale, dans laquelle la dose de nitrate est de 0,01 à 10 mmol de nitrate de sodium/kg de poids corporel/jour, la dose de nitrite est de 0,001 à 1 mmol/kg de poids corporel/jour, et dans laquelle l'intervalle de rapport de dose lorsque le nitrate et le nitrite sont administrés en combinaison est de 5/1 à 100/1 (nitrate/nitrite), dans laquelle ladite performance est améliorée relativement à la performance physique dudit mammifère durant ledit exercice physique en l'absence de ladite administration de nitrate et/ou de nitrite inorganiques.

4. Utilisation selon la revendication 3, dans laquelle ladite composition est sous la forme d'un liquide, d'une pâte, d'une barre, d'un gâteau, d'une poudre, d'un granule, d'un comprimé effervescent, d'un comprimé, d'une gélule, d'une pastille, d'un chewing-gum, d'un comprimé ou d'un cachet à dissolution rapide, d'un comprimé sublingual ou d'une pulvérisation.

5. Utilisation selon l'une quelconque des revendications 3 à 4, dans laquelle ladite composition est sous la forme d'une boisson pour sportifs, d'une boisson énergétique, d'une barre pour sportifs, ou d'une barre énergétique.

6. Utilisation selon l'une quelconque des revendications 3 à 5, dans laquelle la source de nitrate inorganique est choisie parmi un concentré, un extrait ou un jus de légumes contenant du nitrate ou un sel de nitrate inorganique.

7. Utilisation selon l'une quelconque des revendications 3 à 6, dans laquelle ledit nitrate inorganique est utilisé en combinaison avec des polyphénols.

8. Utilisation selon l'une quelconque des revendications 3 à 7, dans laquelle ledit nitrate inorganique est utilisé en combinaison avec des bactéries vivantes capables d'améliorer la réduction du nitrate ou du nitrite.

9. Utilisation selon l'une quelconque des revendications 3 à 8, dans laquelle ledit nitrate inorganique est utilisé en combinaison avec de l'éthanol en une quantité inférieure à 5 % (v/v), de préférence inférieure à 2 % (v/v), de manière davantage préférée de 0,5 à 1 % (v/v).

FIGURE 1

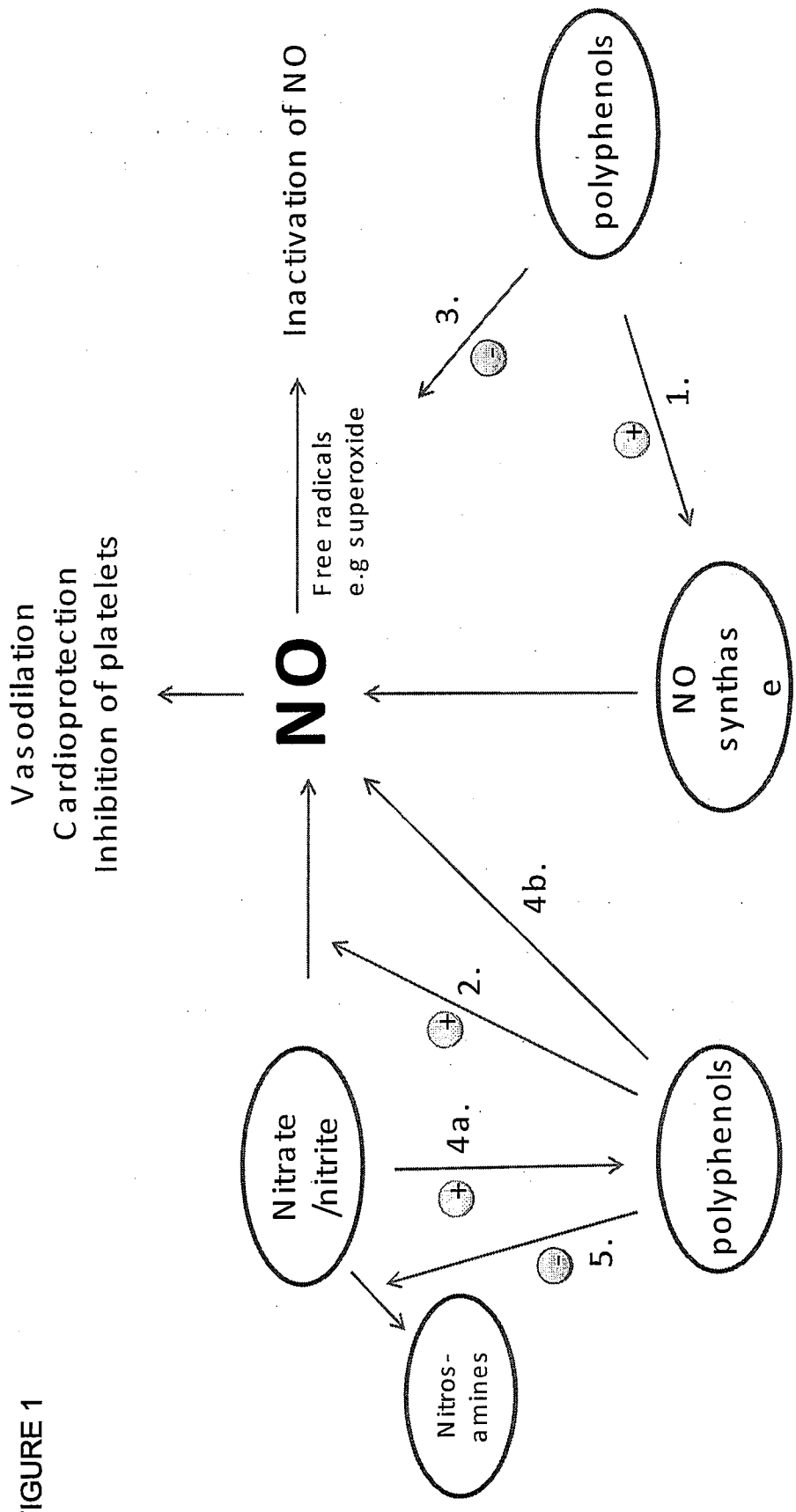

Vasodilation
Cardioprotection
Inhibition of platelets
NO
Free radicals
e.g superoxide
Inactivation of NO
polyphenols
NO synthase
Nitrate /nitrite
Nitros-amines
polyphenols
1.
2.
3.
4a.
4b.
5.

FIGURE 2

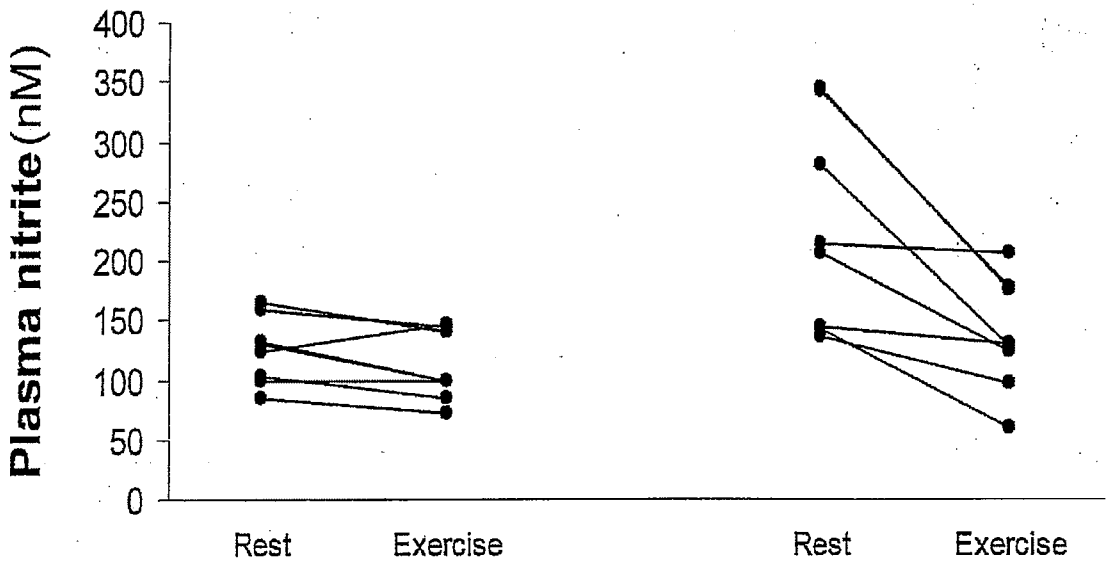

Plasma nitrite (nM)
400
350
300
250
200
150
100
50
0
Rest
Exercise
Rest
Exercise

FIGURE 3

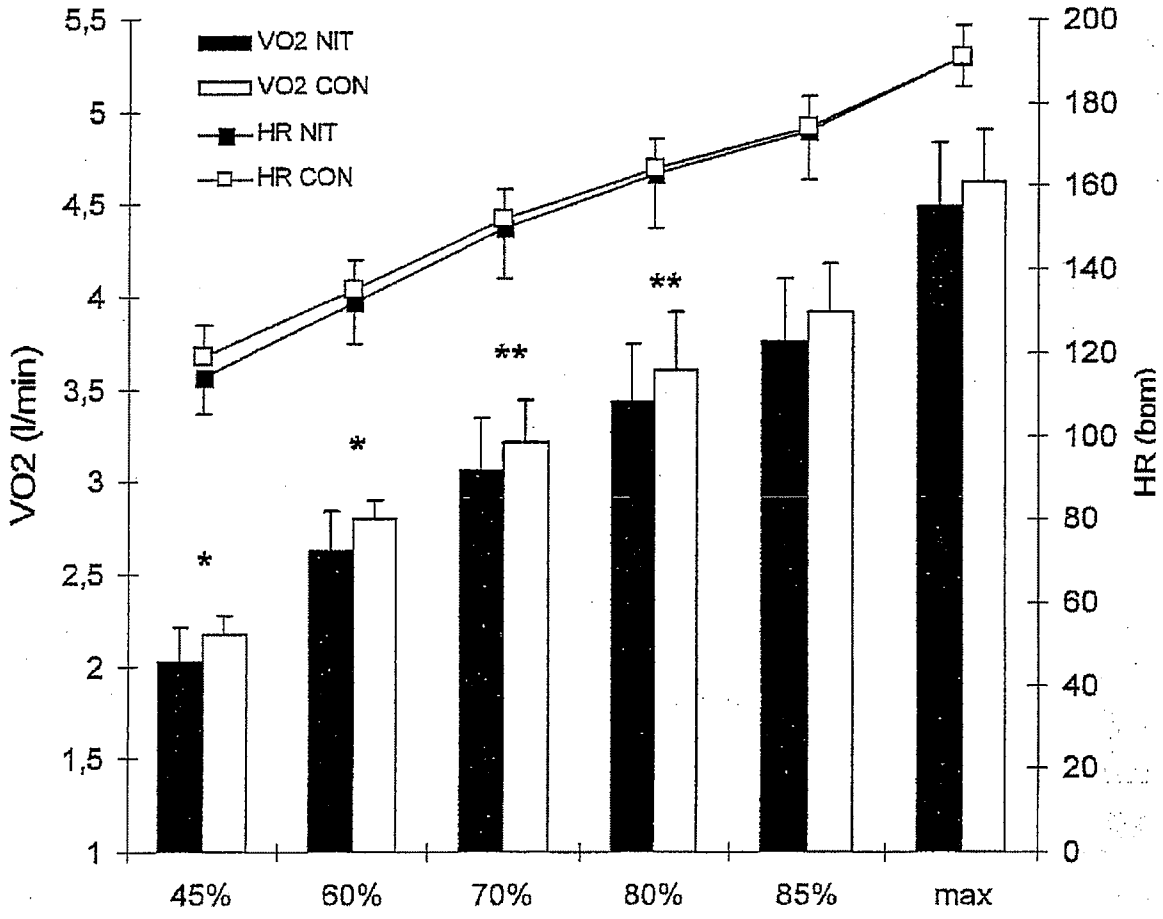

VO2 NIT
VO2 CON
HR NIT
HR CON
VO2 (l/min)
HR (bpm)
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
200
180
160
140
120
100
80
60
40
20
0
*
*
**
**
45%
60%
70%
80%
85%
max

FIGURE 4

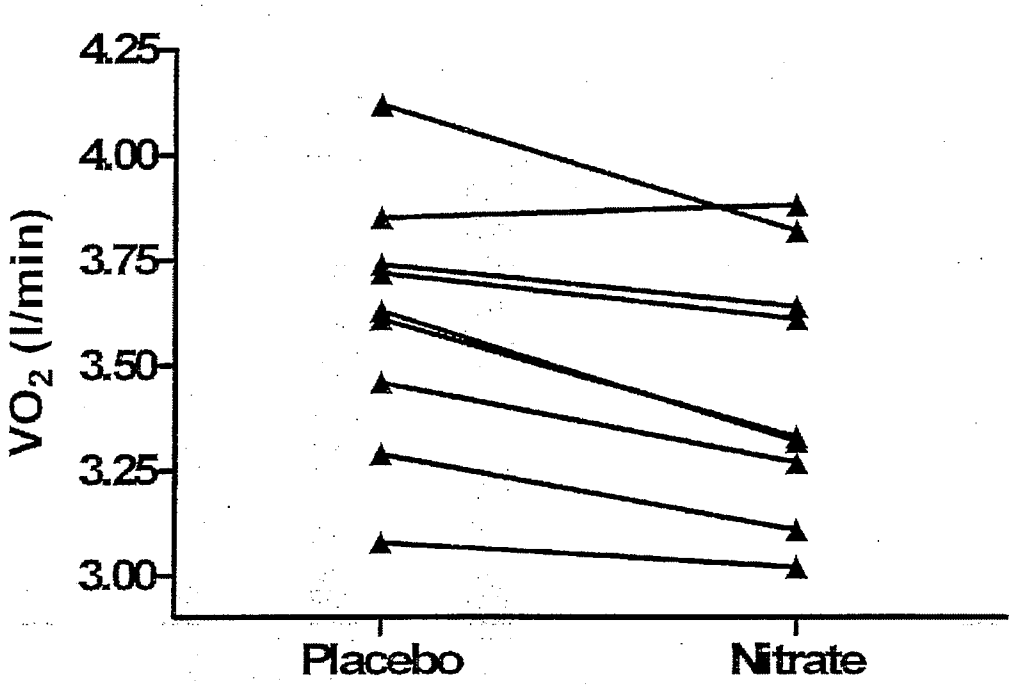
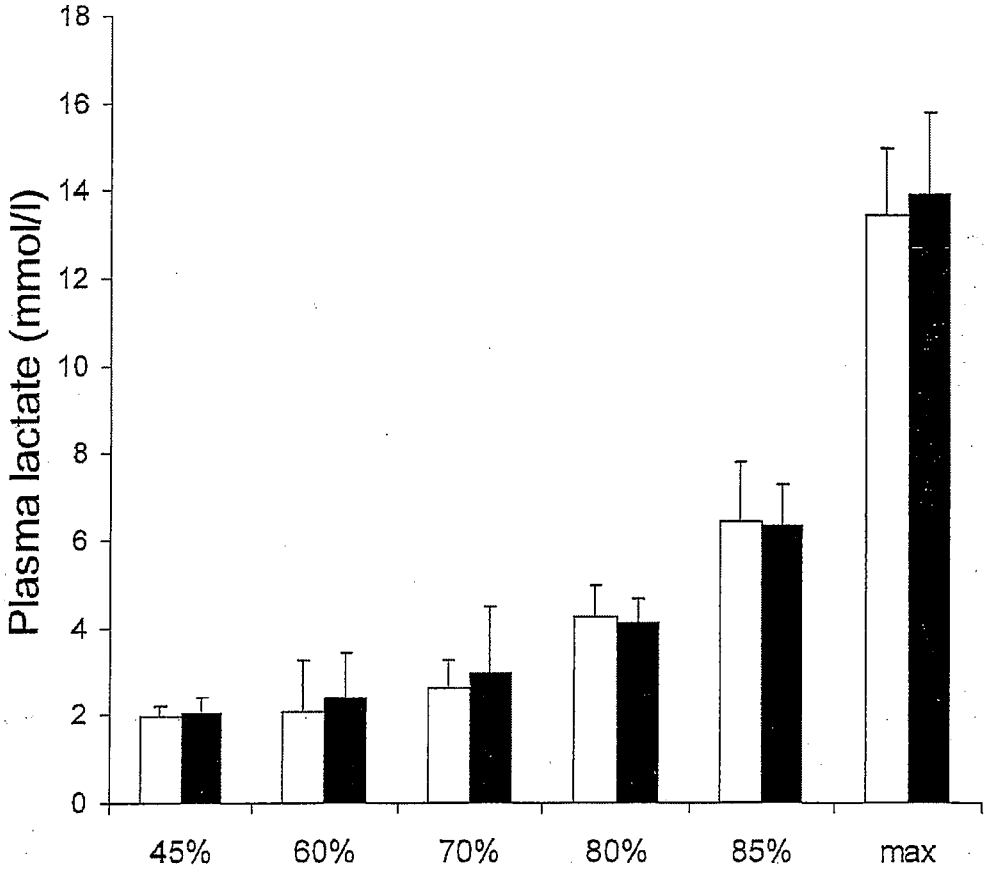


FIGURE 5



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SZABADALMI IGÉNYPONTOK

1. Eljárás egy emlős teljesítményének nem terápiás fokozására, ahol az említett fokozott teljesítmény fizikai terhelésnél az oxigén fogyasztás (VO_2) csökkenésében jelentkezik, azzal jellemezve, hogy szervetlen nitrátot, nitritet vagy ezek kombinációját adagoljuk orálisan, ahol a nitrát dózis 0,01-10 mmol nátrium-nitrát/kg testtömeg/nap, a nitrit dózis 0,001-1 mmol/kg testtömeg/nap, és ahol nitrát és nitrit kombinált adagolása esetén a dózis arány intervallum 5:1-100:1 (nitrát:nitrit), ahol az említett teljesítmény az említett emlős olyan fizikai terhelés alatt jelentkező fizikai teljesítményéhez viszonyítva fokozódik, amelynél nem történt szervetlen nitrát és/vagy nitrit adagolása.

2. Az 1. igénypont szerinti eljárás, ahol az említett nitrátot és/vagy nitritet polifenolokkal együtt adagoljuk.

3. Szervetlen nitrát vagy nitrit vagy ezek kombinációja alkalmazása egy emlős teljesítményének nem terápiás fokozására, ahol az említett fokozott teljesítmény fizikai terhelésnél az oxigén fogyasztás (VO_2) csökkenésében jelentkezik, azzal jellemezve, hogy szervetlen nitrátot, nitritet vagy ezek kombinációját adagoljuk orálisan, ahol a nitrát dózis 0,01-10 mmol nátrium-nitrát/kg testtömeg/nap, a nitrit dózis 0,001-1 mmol/kg testtömeg/nap, és ahol nitrát és nitrit kombinált adagolása esetén a dózis arány intervallum 5:1-100:1 (nitrát:nitrit), ahol az említett teljesítmény az említett emlős olyan fizikai terhelés alatt jelentkező fizikai teljesítményéhez viszonyítva fokozódik, amelynél nem történt szervetlen nitrát és/vagy nitrit adagolása.

4. A 3. igénypont szerinti alkalmazás, ahol az említett készítmény formája folyadék, paszta, pálca/rúd, tábla, por, granulátum, pezsgőtabletta, tabletta, kapszula, szopogatós tabletta, rágógumi, gyorsan olvadó tabletta vagy ostya, szublingualis tabletta vagy spray.

5. A 3-4. igénypontok bármelyike szerinti alkalmazás, ahol az említett készítmény formája sport ital, energia ital, sport pálca/rúd vagy energia pálca/rúd.

6. A 3-5. igénypontok bármelyike szerinti alkalmazás, ahol a szervetlen nitrát forrása koncentrátum, nitrátot tartalmazó zöldség extraktuma vagy leve, vagy szervetlen nitrátsó közül megválasztott.

7. A 3-6. igénypontok bármelyike szerinti alkalmazás, ahol az említett szervetlen nitrátot polifenolokkal kombinálva alkalmazzuk.

8. A 3-7. igénypontok bármelyike szerinti alkalmazás, ahol az említett szervetlen nitrátot nitrát vagy nitrit redukcióját fokozni képes élő baktériummal kombinálva alkalmazzuk.

9. A 3-8. igénypontok bármelyike szerinti alkalmazás, ahol az említett szervetlen nitrátot etanollal 3 tf/tf% alatti, előnyösen 2 tf/tf% alatti, különösen előnyösen 0,5-1 tf/tf% közötti mennyiségben kombinálva alkalmazzuk.