COMPOSITIONS OF NITRATES AND METHODS OF USE THEREOF

Related Applications

[0001] This application is a continuation application of earlier U.S. Utility Patent Application to Jon Lundberg and Eddie Weitzberg, titled "Compositions of Nitrates and Methods of Use Thereof," application serial number 15/966,508, filed April 30, 2018, now pending, which is a continuation application of earlier U.S. Utility Patent Application to Jon Lundberg and Eddie Weitzberg, titled "Use of Nitrites and Nitrates and Compositions Containing These," application serial number 12/528,794, filed June 17, 2013, now pending, which is a national stage application of PCT application No. PCT/SE08/50212, filed February 26, 2008, which claims the benefit of the filing date of U.S. Provisional Patent Application 60/919,709 to Jon Lundberg and Eddie Weitzberg, filed on March 22, 2007, the disclosures of all of which being hereby incorporated entirely herein by reference.

[0002] U.S. Patent Application No. 15/966,508 is also a continuation application of earlier U.S. Utility Patent Application to Jon Lundberg and Eddie Weitzberg, titled "Performance Enhancing Compositions and Use Thereof," U.S. Patent Application No. 14/830,937, filed August 20, 2015, now pending, which is a continuation application of U.S. Patent Application No. 12/528,798, filed August 26, 2009, now issued as U.S. Patent No. 9,180,140, which was a national stage application of PCT application No. PCT/SE 2008/050211, filed February 26, 2008, which claims priority to Swedish Patent Application 0700520-0, which was filed February 26, 2007, and Swedish Patent Application 0700729-0, which was filed March 22, 2007, the disclosures of all of which being hereby incorporated entirely herein by reference.

Field of the Invention

[0003] 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. The present invention also relates to the field of medicine and pharmaceuticals, in particular pharmaceuticals and therapeutic methods for lowering metabolic rate, oxygen consumption and/or glucose homeostasis in a human patient or another mammal, based on the administration of nitrates and/or nitrites to said patient or mammal.

Background of the Invention

[0004] 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). 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.sub.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.

[0005] Recent studies indicate that nitrate and nitrite can have significant biological effects in the body and that these effects may be beneficial (LUNDBERG, Jon O., et al. Nitrate, becteria and human health. Nat Rev Microbiol. 2004, no. 2, p. 593-602). For example, the nitrite anion can cause vasodilatation at near physiological concentrations when tested in vitro (MODIN, A., et al. Nitrite-derived nitric oxide: a possible mediator of `acidic-metabolic` vasodilation. Acta Physiol Scand. 2001, vol. 171, p. 9-16) or when infused intra-arterially to humans (COSBY, K., et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med. 2003, no. 9, p. 1498-505). Nitrate can be converted to nitrite in vivo in a process dependent on commensal bacteria (SPIEGELHALDER, B., et al. Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. Food Cosmet Toxicol. 1976, no. 14, p. 545-548). When nitrate is ingested it is rapidly absorbed into blood and then accumulates in saliva. In the oral cavity bacteria reduce parts of the dietary nitrate to nitrite and nitrite can then enter the systemic circulation. (LUNDBERG, Jon O., et al. Inorganic nitrate is a possible source for systemic generation of nitric oxide. Free Radic Biol Med. 2004, vol. 37, p. 395-400).

[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). 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. To date the focus among researchers has been on the cardiovascular effects of nitrite after its in vivo reduction to the vasodilator nitric oxide (NO) (COSBY et al. (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, p. 1232-1240; GLADWIN, M. T., et al. The emerging biology of the nitrite anion. Nat Chem Biol. 2005, no. 1, p. 308-14; LARSEN, F. J., et al. Effects of dietary nitrate on blood pressure in healthy volunteers. N Engl J Med. 2006, vol. 355, p. 2792-3). WO 2005/004884 A (US GOVERNMENT ET AL.) 2005-01-20 and WO 2005/007173 A (US GOVERNMENT ET AL.) 2005-01-27 describe a method to administer a nitrite salt specifically to obtain vasodilatation in a subject. No effects of low-dose nitrate/nitrite on energy expenditure or glucose homeostasis or the effects of NO on cellular respiration during physical exercise have been described.

[0007] Physiological adaptation to exercise 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).

[0008] 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. 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.

[0009] 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 Jungersten's 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] Nitrate (NO_3^-) and nitrite (NO_2^-) are generally viewed as unwanted residues in the food chain with potentially harmful effects (Joint FAO/WHO Expert Committee on Food Additives (JECFA). Safety Evaluation of Certain Food Additives. WHO, 1970. ISBN 9241660503; TANNENBAUM, S. R., et al. Nitrite in human saliva. Its possible relationship to nitrosamine formation. J cancer Ins. 1974, vol. 53, p. 79-84; BARTSCH, H., et al. Inhibitors of endogenous nitrosation: mechanisms and implications in human cancer prevention. Mutation Res. 1988, vol. 202, p. 307-324). Proposed harmful effects of these anions include promotion of gastric cancers and other malignancies and development of methemoglobinemia in infants. Because of this the levels of nitrate/nitrite are strictly regulated in food and drinking water.

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