

Review Article

Is Nitric Oxide a Hormone?

Asghar Ghasemi and Saleh Zahediasl*

Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti
University of Medical Sciences, Tehran, Iran

Received 11 May 2011; revised 31 May 2011; accepted 15 June 2011

ABSTRACT

Nitric oxide (NO) is a simple ubiquitous signaling molecule and plays important roles in almost every biological system. Recent evidences suggest that NO may act as an endocrine molecule. The aim of this review is considering available literature on endocrine roles of NO and/or its metabolites, i.e. nitrite and nitrate. Existing data suggest the idea that NO is a hormone that after production in tissues, it is stabilized and transported as nitrite and/or S-nitrosothiols in the blood to target cells. *Iran. Biomed. J.* 15 (3): 59-65, 2011

Keywords: Endocrine, Hormone, Nitric oxide (NO), Nitrate, Nitrite

HISTORY

Nitric oxide (NO) was discovered in 1772 [1]. Nitroglycerine (NG), a vasodilator acting via NO production, was synthesized in 1847 [2, 3]. The effect of NG was studied on healthy volunteers by Constantin Hering in 1849 and it was proven to cause headache [2]. Later in 1878, NG was used by William Murrell for the first time to treat angina [2]. Towards the end of 19th century, NG was established as a remedy for relief of anginal pain [2]. In 1916, Mitchell *et al.* [4] suggested that body tissues can also produce nitrate and Richard Bodo [5] in 1928 showed a dose-dependent increase of coronary flow in response to sodium nitrite administration. In 1970s, it was shown that nitrite-containing compounds stimulate guanylate cyclase and increase cyclic guanosine monophosphate (cGMP) which causes vascular relaxation and it is presumed that cGMP activation may occur via the formation of NO [2].

In 1980, Furchgott and Zawadzki [6] showed that endothelial cells are required for acetylcholine-induced relaxation of vascular bed through the endothelium-derived relaxing factor. Thereafter in 1987, it was shown that endothelium-derived relaxing factor and NO are the same or almost the same [7-9]. In 1992, NO was proclaimed as the molecule of the year [10] and in 1999, Furchgott, Ignarro, and Murad were awarded the

Nobel Prize in Physiology or Medicine for studies in the NO field [1]. Due to the proven roles played by NO physiologically and pathologically, research on NO was increased rapidly and at the end of 20th century, the rate of NO publications was approximately 6,000 papers per year [1], with currently more than 100,000 references invoking NO listed in PubMed.

NO synthesis. NO is produced in all tissues [11] and the general belief is that its local production determines physiological actions [12-14].

Enzymatic and non-enzymatic NO synthesis. NO is synthesized from L-arginine by the enzymes known as NO synthase (NOS) (EC 1.14.13.39) in two separate mono-oxygenation steps; first, L-arginine is converted to N^ω-hydroxyarginine in a reaction requiring one O₂ and one NADPH and the presence of tetrahydrobiopterin (BH₄) and in the second step, by oxidation of N^ω-hydroxyarginine citrulline and NO are formed [15]. At least three NOS enzyme isoforms including neuronal, inducible, and endothelial (eNOS) have been identified and encoded by different genes [16-18]. In 1997, Ghafourifar and Richter [19] suggested the existence of mitochondrial NOS and in 1994, Lundberg and colleagues [20] and Benjamin and colleagues [21] demonstrated NOS-independent NO formation. Non-enzymatic NO production by one-

*Corresponding Author; Tel.: (+98-21) 2240 9309; Fax: (+98-21) 2240 2463; E-mail: zahedi@endocrine.ac.ir

electron reduction of nitrite, a blood and tissue NO reservoir [22], seems to be ubiquitous and greatly accelerated under hypoxic conditions [23]. This finding changes the general belief that nitrate and nitrite are waste products of NO [24].

Rate of NO formation. The rate of NO formation differs between species, 0.33-0.85 $\mu\text{mol/kg/h}$ in Wistar rats [25], 7.68 ± 1.47 in C57/B16 mice [26] and 0.9 $\mu\text{mol/kg/h}$ [26] or about 1 mmol/day [27, 28] in humans. The storage form of NO in tissues is not so high [28] and in conditions such as hypoxia, ischemia, or injury in which the L-arginine/NOS pathway is impaired, consumption of serum NO_x (nitrite + nitrate = NO_x) seems to produce NO [29, 30].

NO metabolites. Rates of formation and clearance of NO determine its steady state concentration [28]. The NO half-life *in vivo* in the circulation is most likely shorter than 0.1 s [31]. The major breakdown product of NO in aqueous solution, free of biological material, is nitrite [28, 32] while in the presence of sufficient amount of O_2 it is nitrate [28]. In plasma, NO is oxidized almost completely to nitrite, where it remains stable for several hours [28]. In whole blood, nitrite is rapidly converted to nitrate [28], and NO reacts with oxyhemoglobin to produce methemoglobin and nitrate [32]. In blood, NO is taken up by red blood cells and converted to nitrate, a major metabolic pathway for endogenously produced NO [32]. In the body, 90% NO is converted to nitrate, which is the main stable end product of NO formation *in vivo* [25, 33]. The half-lives of nitrate and nitrites in circulation are 5-8 h and 110 s, respectively [34, 35]. Because of short half-life of NO itself, most often its serum metabolites are measured as a surrogate for NO production [12, 36, 37]. Serum/plasma NO_x levels, the most suitable method to assess NO synthesis *in vivo* [32], are highly correlated with endogenous NO production [38]. Nitrate concentration in the blood is a major factor in determining the nitrate and nitrite levels of the rest of the body [27]. Reference values for serum NO_x concentration have been reported in both adults [39] and pediatrics [40].

Physiological roles of NO. NO is known as a ubiquitous, an omnipresent and a pleiotropic signaling molecule [18, 41, 42] and plays important roles in almost every biological system [17, 43]. It is a potent vasodilator and considered as a key regulator in vascular homeostasis by inhibition of platelet activation, inhibition of vascular smooth muscle proliferation, and control of blood pressure [44-46]. Neural transmission [47-50], memory [44], apoptosis [51], reproduction [52], lipolysis [53], regulation of energy balance [42], and host defense [48] are among

other physiological processes where NO plays some roles. NO regulates hormone release in the hypothalamic-pituitary axis [49] and inhibits prolactin secretion [54] and may play a role in catecholamine release and steroidogenesis in adrenal gland [48] and can regulate actions of insulin and carbohydrate metabolism [55, 56]. NO also has a role in thyrocyte/thyroid function and sodium nitroprusside, a precursor of NO, increases cGMP in human thyrocytes [54, 57]. Estrogens increase the synthesis and release of NO through stimulation of eNOS gene expression [58] and it has been suggested that enhancement of NO activity is a mechanism for attenuation arterial hypertension in females [54, 59].

NO and metabolic/endocrine disorders. Today, it has been revealed that many diseases are associated with altered NO homeostasis [1]. Endothelial dysfunction, which is related to all cardiovascular risk factors, now has become synonymous with reduced biological activity of NO and is considered as a hallmark of cardiovascular disease [1, 46]. Several studies have shown an association between serum/plasma NO_x levels as an index for NO generation [12, 31, 60] and eNOS activity [34, 61], and different diseases including diabetes and dysglycemia [62-67], thyroid disorders [68], metabolic syndrome [63, 69-71], hypertension [72-74], heart failure [75] cardiovascular disease [11], and obesity [39, 76, 77]. Defects in endogenous synthesis and bioavailability of NO have been proposed as a common underlying molecular mechanism linking metabolic and cardiovascular disease [78] and serum NO_x level has been loaded with other metabolic syndrome components in the cluster analysis, suggesting a unifying role in the clustering of MetS components [71]. NO deficiency, which is related to hyperinsulinemia, affects glucose and lipid metabolism and seems to be a link between metabolic and cardiovascular disease [54]. In addition, it has been shown that excessive NO production impairs β cell function causing death [79]; while inducible NOS is involved in muscle insulin resistance [80] lack of eNOS causes insulin resistance [81]. Impairment of NO synthesis may represent a central defect causing metabolic abnormalities associated with insulin resistance [82]. All three isoforms of NOS are targets for thyroid hormones [83]. In human hyperthyroidism, increased NO production plays a role in vasodilation and abnormal vascular tone [84]. One study has reported impaired NO production in newborns with primary congenital hypothyroidism [85]. NOS activity is upregulated in hyperthyroid rats and may participate in cardiovascular manifestation of the disease, while in hypothyroid rats, the tissue response to NOS activity is heterogeneous [86].

Protective effects of nitrite and nitrate. It has been shown that 3-day dietary supplementation with sodium nitrate (0.1 mmol/kg/day) could reduce significantly diastolic blood pressure in non-smoking healthy volunteers [87]. Recently, a large cohort study of 52,693 patients from 14 countries with acute coronary syndrome, of whom 20% were on chronic nitrate, demonstrated that chronic nitrate therapy (medication routinely took at home and started at least 7 days prior to index event) was associated with reduced severity of myocardial injury in response to acute coronary events [88]. The result showed that the proportion of these subjects with ST-segment elevation myocardial infarction was 41% in nitrate-naïve patients compared to only 18% in nitrate users and conversely a higher percent of nitrate users (82%) presented with non-ST-segment elevation acute coronary syndrome compared to 59% in nitrate-naïve patients [88].

Increasing nitrate or nitrate dietary intake provides significant cardioprotection against ischemia-reperfusion (I/R) injury in mice and it has been proposed that nitrite-/nitrate-rich foods may provide protection against cardiovascular conditions characterized by ischemia [89]. It has been suggested that the nitrate-nitrite-NO pathway serves as a backup system to ensure sufficient NO generation under hypoxic condition when NOS may be malfunctioning [23].

Abundant consumption of fruits and vegetables, especially green leafy vegetables, is associated with lower risk of cardiovascular disease [24]. It has been proposed that inorganic nitrate, which is found in vegetables with a high concentrations, i.e. >2000-3000 mg/nitrate/kg [90], is the major factor in contributing to the positive health effects of vegetables via bioconversion to nitrite, NO, and nitroso-compounds [24], NO_x intake now being considered as a dietary parameter for assessing cardiovascular risk [89].

Any intervention that increases blood and tissue concentration of nitrite may provide cardioprotection against I/R injury because it serves as a NOS-independent source of NO and reacts with thiols to form S-nitrosothiols [89]. Nitrate-nitrite-NO pathway can be boosted by exogenous administration of nitrate or nitrite and this may have important therapeutic as well as nutritional implications [23]. However, additional studies are required to clarify the protective roles of nitrate, considering the medical status of subjects, concomitant use of inhibitors of endogenous nitrosation (e.g. vitamin C and E), or foods containing high levels of nitrosatable precursors (e.g. fish) [91, 92]. Some individuals, including those with high blood pressure and atherosclerosis may benefit from increased nitrate while those with esophageal dysplasia should avoid foods with high concentration of nitrate [93].

Adverse health effects of nitrate and nitrite. NO_x has generally been regarded as a harmful substance leading to the enactment of strict limits on concentration of nitrate and nitrite in drinking water [89, 94]; 50 mg/L for nitrate and 3 mg/L for nitrite. The sum of the ratios of the concentration of each to its guideline value should not exceed 1 [95]; however, some authors believe that these limits are overprotective [90], while others do not [91]. Two health issues that have been attributed to nitrate are cancers of digestive tract and infant methaemoglobinaemia [30, 94]. Quite number of epidemiological studies, carried out during the last four decades, concluded that no convincing link between nitrate and stomach cancer incidence and mortality can be established [94]; neither has any causative link between nitrite or nitrate exposure and cancer been documented [89]. Therefore, it has been suggested that none of the health claims against dietary nitrate are substantiated [90] and it is the time to double the maximum contaminant level of nitrate, based on evidence [90]. Nevertheless, it must be notified there are some disagreements on this decision [91].

Evidences for an endocrine role of NO. The first evidence for intravascular NO transport, provided by Cannon III *et al.* [96] in 2001, showed that inhaled NO can be transported in the blood and results in peripheral vasodilation. In 2002, Rassaf *et al.* [97] using intra-arterial aqueous NO solution suggested for the first time that NO is transported considerable distances in human plasma; and they criticized the ultra-short half-life of NO in blood which is reported to be 0.05 -1.8 ms [28]. The authors suggested that longer activation of the downstream signaling cascade, formation of intermediates, or reduced NO consumption under flow condition may be likely explanations that lifespan of NO in plasma lies with the seconds to minutes [97]. In 2003, Schechter and Gladwin [98] presented the hypothesis that "NO may be transported throughout the body in the manner of a hormone". In 2008, Elrod *et al.* [99] showed that eNOS-generated NO in the heart of mice, with cardiac-specific overexpression of human eNOS gene, is transported in plasma and protects the liver against I/R injury; suggesting that NO can exert endocrine activity.

NO has a short half-life in the blood that limits its transport to distant organs [31, 99]; therefore, it has been suggested that nitrite [32, 99-102] and/or S-nitrosothiol [32, 97, 103-105] are responsible for intravascular transport of NO. To support endocrine roles for NO, it has been shown that inhaled NO increases plasma levels of nitrate in healthy subjects by ≈ 50% [33], produces diuretic actions and produces extrapulmonary systemic vascular effects [101, 106, 107]. In addition, dietary nitrite supplementation

protects mice against myocardial I/R injury [89]. Recently, Carlstrom *et al.* [78], have shown that dietary nitrate supplementation in eNOS-deficient mice can partly compensate for disturbances in endogenous eNOS NO generation and attenuates features of metabolic syndrome. Finally, it has been proposed recently that a nitrite/nitrate/NO endocrine system along with NOS enzymes help to maintain NO bioavailability [108].

CONCLUSION

Existing data suggest and support this idea that NO is a hormone, which after its production in tissues, is stabilized and transported as nitrite and/or S-nitrosothiols in the blood to target cells. Therefore, further studies are required to explore the endocrine roles of NO.

REFERENCES

1. Yetik-Anacak, G. and Catravas, J.D. (2006) Nitric oxide and the endothelium: history and impact on cardiovascular disease. *Vascul. Pharmacol.* 45: 268-276.
2. Marsh, N. and Marsh, A. (2000) A short history of nitroglycerine and nitric oxide in pharmacology and physiology. *Clin. Exp. Pharmacol. Physiol.* 27: 313-319.
3. Ignarro, L.J. (2002) After 130 years, the molecular mechanism of action of nitroglycerin is revealed. *Proc. Natl. Acad. Sci. USA.* 99: 7816-7817.
4. Mitchell, H.H., Shonle, H.A. and Grindley, H.S. (1916) The origin of the nitrates in the urine. *J. Biol. Chem.* 24: 461-490.
5. Bodo, R. (1928) The effect of the "heart-tonics" and other drugs upon the heart-tone and coronary circulation. *J. Physiol.* 64: 365-387.
6. Furchgott, R.F. and Zawadzki, J.V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373-376.
7. Ignarro, L.J., Buga, G.M., Wood, K.S., Byrns, R.E. and Chaudhuri, G. (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. USA.* 84: 9265-9269.
8. Palmer, R.M., Ferrige, A.G. and Moncada, S. (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327: 524-526.
9. Furchgott, R.F. (1995) A research trail over half a century. *Annu. Rev. Pharmacol. Toxicol.* 35: 1-27.
10. Koshland, D.E., Jr. (1992) The molecule of the year. *Science* 258: 1861.
11. Higashino, H., Tabuchi, M., Yamagata, S., Kurita, T., Miya, H., Mukai, H. and Miya, Y. (2010) Serum nitric oxide metabolite levels in groups of patients with various diseases in comparison of healthy control subjects *J. Med. Sci.* 10: 1-11.
12. Baylis, C. and Vallance, P. (1998) Measurement of nitrite and nitrate levels in plasma and urine--what does this measure tell us about the activity of the endogenous nitric oxide system? *Curr. Opin. Nephrol. Hypertens.* 7: 59-62.
13. Lundberg, J.O. and Govoni, M. (2004) Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic. Biol. Med.* 37: 395-400.
14. Baylis, C. (2008) Nitric oxide deficiency in chronic kidney disease. *Am. J. Physiol. Renal. Physiol.* 294: F1-9.
15. Knowles, R.G. and Moncada, S. (1994) Nitric oxide synthases in mammals. *Biochem. J.* 298 (Pt 2): 249-258.
16. Wu, G. and Meininger, C.J. (2002) Regulation of nitric oxide synthesis by dietary factors. *Annu. Rev. Nutr.* 22: 61-86.
17. Dudzinski, D.M., Igarashi, J., Greif, D. and Michel, T. (2006) The regulation and pharmacology of endothelial nitric oxide synthase. *Annu. Rev. Pharmacol. Toxicol.* 46: 235-276.
18. Pacher, P., Beckman, J.S. and Liaudet, L. (2007) Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.* 87: 315-424.
19. Ghafourifar, P. and Richter, C. (1997) Nitric oxide synthase activity in mitochondria. *FEBS Lett.* 418: 291-296.
20. Lundberg, J.O., Weitzberg, E., Lundberg, J.M. and Alving, K. (1994) Intragastric nitric oxide production in humans: measurements in expelled air. *Gut* 35: 1543-1546.
21. Benjamin, N., O'Driscoll, F., Dougall, H., Duncan, C., Smith, L., Golden, M. and McKenzie, H. (1994) Stomach NO synthesis. *Nature* 368: 502.
22. Raat, N.J., Noguchi, A.C., Liu, V.B., Raghavachari, N., Liu, D., Xu, X., Shiva, S., Munson, P.J. and Gladwin, M.T. (2009) Dietary nitrate and nitrite modulate blood and organ nitrite and the cellular ischemic stress response. *Free Radic. Biol. Med.* 47: 510-517.
23. Lundberg, J.O. and Weitzberg, E. (2010) The biological role of nitrate and nitrite: the times they are a-changin'. *Nitric Oxide* 22: 61-63.
24. Lundberg, J.O., Feelisch, M., Bjorne, H., Jansson, E.A. and Weitzberg, E. (2006) Cardioprotective effects of vegetables: is nitrate the answer? *Nitric Oxide* 15: 359-362.
25. Sakinis, A. and Wennmalm, A. (1998) Estimation of total rate of formation of nitric oxide in the rat. *Biochem. J.* 330 (Pt 1): 527-532.
26. Wickman, A., Klintland, N., Gan, L.M., Sakinis, A., Soderling, A.S., Bergstrom, G. and Caidahl, K. (2003) A technique to estimate the rate of whole body nitric oxide formation in conscious mice. *Nitric Oxide* 9: 77-85.
27. Wagner, D.A., Schultz, D.S., Deen, W.M., Young, V.R. and Tannenbaum, S.R. (1983) Metabolic fate of an oral dose of 15N-labeled nitrate in humans: effect of diet supplementation with ascorbic acid. *Cancer Res.* 43: 1921-1925.
28. Kelm, M. (1999) Nitric oxide metabolism and breakdown. *Biochim. Biophys. Acta.* 1411: 273-289.

29. Lopez-Ramos, J.C., Martinez-Romero, R., Molina, F., Canuelo, A., Martinez-Lara, E., Siles, E. and Peinado, M.A. (2005) Evidence of a decrease in nitric oxide-storage molecules following acute hypoxia and/or hypobaria, by means of chemiluminescence analysis. *Nitric Oxide* 13: 62-67.
30. Alef, M.J., Vallabhaneni, R., Carchman, E., Morris, S.M., Jr., Shiva, S., Wang, Y., Kelley, E.E., Tarpey, M.M., Gladwin, M.T., Tzeng, E. and Zuckerbraun, B.S. (2011) Nitrite-generated NO circumvents dysregulated arginine/NOS signaling to protect against intimal hyperplasia in Sprague-Dawley rats. *J. Clin. Invest.* 121: 1646-1656.
31. Tsikas, D. (2005) Methods of quantitative analysis of the nitric oxide metabolites nitrite and nitrate in human biological fluids. *Free Radic. Res.* 39: 797-815.
32. Tsikas, D. (2007) Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 851: 51-70.
33. Wennmalm, A., Benthin, G., Edlund, A., Jungersten, L., Kieler-Jensen, N., Lundin, S., Westfelt, U.N., Petersson, A.S. and Waagstein, F. (1993) Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. *Circ. Res.* 73: 1121-1127.
34. Bryan, N.S. and Grisham, M.B. (2007) Methods to detect nitric oxide and its metabolites in biological samples. *Free Radic. Biol. Med.* 43: 645-657.
35. Watanabe, T., Akishita, M., Toba, K., Kozaki, K., Eto, M., Sugimoto, N., Kiuchi, T., Hashimoto, M., Shirakawa, W. and Ouchi, Y. (2000) Influence of sex and age on serum nitrite/nitrate concentration in healthy subjects. *Clinica. Chimica. Acta.* 301: 169-179.
36. Moshage, H., Kok, B., Huizenga, J.R. and Jansen, P.L. (1995) Nitrite and nitrate determinations in plasma: a critical evaluation. *Clin. Chem.* 41: 892-896.
37. Ghasemi, A., Hedayati, M. and Biabani, H. (2007) Protein precipitation methods evaluated for determination of serum nitric oxide end products by the Griess assay. *J. Med. Sci. Res.* 2: 43-46.
38. Sastry, K.V., Moudgal, R.P., Mohan, J., Tyagi, J.S. and Rao, G.S. (2002) Spectrophotometric determination of serum nitrite and nitrate by copper-cadmium alloy. *Anal. Biochem.* 306: 79-82.
39. Ghasemi, A., Zahediasl, S. and Azizi, F. (2010) Reference values for serum nitric oxide metabolites in an adult population. *Clin. Biochem.* 43: 89-94.
40. Ghasemi, A., Zahediasl, S. and Azizi, F. (2010) Reference values for serum nitric oxide metabolites in pediatrics. *Nitric Oxide* 23: 264-268.
41. Stuart-Smith, K. (2002) Demystified. Nitric oxide. *Mol. Pathol.* 55: 360-366.
42. Joost, H.G. and Tschop, M.H. (2007) NO to obesity: does nitric oxide regulate fat oxidation and insulin sensitivity? *Endocrinology* 148: 4545-4547.
43. Yoon, S., Moon, J., Shin, C., Kim, E., Jo, S.A. and Jo, I. (2002) Smoking status-dependent association of the 27-bp repeat polymorphism in intron 4 of endothelial nitric oxide synthase gene with plasma nitric oxide concentrations. *Clinica. Chimica. Acta.* 324: 113-120.
44. Moncada, S. (1999) Nitric oxide: discovery and impact on clinical medicine. *J. R. Soc. Med.* 92: 164-169.
45. Cannon, R.O. 3rd. (1998) Role of nitric oxide in cardiovascular disease: focus on the endothelium. *Clin. Chem.* 44: 1809-1819.
46. Pandolfi, A. and Di Pietro, N. (2010) High glucose, nitric oxide, and adenosine: a vicious circle in chronic hyperglycaemia? *Cardiovasc. Res.* 86: 9-11.
47. Garthwaite, J., Charles, S.L. and Chess-Williams, R. (1988) Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 336: 385-388.
48. Moncada, S., Palmer, R.M. and Higgs, E.A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43: 109-142.
49. Dawson, T.M. and Dawson, V.L. (1996) Nitric oxide synthase: role as a transmitter/mediator in the brain and endocrine system. *Annu. Rev. Med.* 47: 219-227.
50. Wiesinger, H. (2001) Arginine metabolism and the synthesis of nitric oxide in the nervous system. *Prog. Neurobiol.* 64: 365-391.
51. Bredt, D.S. and Snyder, S.H. (1994) Nitric oxide: a physiologic messenger molecule. *Annu. Rev. Biochem.* 63: 175-195.
52. Inoue, T., Kaibara, M., Sakurai-Yamashita, Y., Kawano, M., Ishimaru, T. and Taniyama, K. (2004) Increases in serum nitrite and nitrate of a few-fold adversely affect the outcome of pregnancy in rats. *J. Pharmacol. Sci.* 95: 228-233.
53. Andersson, K., Gaudiot, N., Ribiere, C., Elizalde, M., Giudicelli, Y. and Arner, P. (1999) A nitric oxide-mediated mechanism regulates lipolysis in human adipose tissue in vivo. *Br. J. Pharmacol.* 126: 1639-1645.
54. Vargas, F., Moreno, J.M., Wangenstein, R., Rodriguez-Gomez, I. and Garcia-Estan, J. (2007) The endocrine system in chronic nitric oxide deficiency. *Eur. J. Endocrinol.* 156: 1-12.
55. Hintze, T.H. (2001) Prologue: Nitric oxide--hormones, metabolism, and function. *Am. J. Physiol. Heart Circ. Physiol.* 281: H2253-2255.
56. Shankar, R.R., Wu, Y., Shen, H.Q., Zhu, J.S. and Baron, A.D. (2000) Mice with gene disruption of both endothelial and neuronal nitric oxide synthase exhibit insulin resistance. *Diabetes* 49: 684-687.
57. Fellet, A.L., Arza, P., Arreche, N., Arranz, C. and Balaszczuk, A.M. (2004) Nitric oxide and thyroid gland: modulation of cardiovascular function in autonomic-blocked anaesthetized rats. *Exp. Physiol.* 89: 303-312.
58. Simoncini, T., Mannella, P., Fornari, L., Caruso, A., Varone, G., Garibaldi, S. and Genazzani, A.R. (2004) Tibolone activates nitric oxide synthesis in human endothelial cells. *J. Clin. Endocrinol. Metab.* 89: 4594-4600.
59. Forte, P., Kneale, B.J., Milne, E., Chowienczyk, P.J., Johnston, A., Benjamin, N. and Ritter, J.M. (1998) Evidence for a difference in nitric oxide biosynthesis between healthy women and men. *Hypertension* 32: 730-734.
60. Miranda, K.M., Espey, M.G. and Wink, D.A. (2001) A

- rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 5: 62-71.
61. Choi, J.W., Pai, S.H., Kim, S.K., Ito, M., Park, C.S. and Cha, Y.N. (2001) Increases in nitric oxide concentrations correlate strongly with body fat in obese humans. *Clin. Chem.* 47: 1106-1109.
 62. Chien, W.Y., Yang, K.D., Eng, H.L., Hu, Y.H., Lee, P.Y., Wang, S.T. and Wang, P.W. (2005) Increased plasma concentration of nitric oxide in type 2 diabetes but not in nondiabetic individuals with insulin resistance. *Diabetes Metab.* 31: 63-68.
 63. Zahedi Asl, S., Ghasemi, A. and Azizi, F. (2008) Serum nitric oxide metabolites in subjects with metabolic syndrome. *Clin. Biochem.* 41: 1342-1347.
 64. Apakkan Aksun, S., Ozmen, B., Ozmen, D., Parildar, Z., Senol, B., Habif, S., Mutaf, I., Turgan, N. and Bayindir, O. (2003) Serum and urinary nitric oxide in Type 2 diabetes with or without microalbuminuria: relation to glomerular hyperfiltration. *J. Diabetes Complications.* 17: 343-348.
 65. Pereira, F.O., Frode, T.S. and Medeiros, Y.S. (2006) Evaluation of tumour necrosis factor alpha, interleukin-2 soluble receptor, nitric oxide metabolites, and lipids as inflammatory markers in type 2 diabetes mellitus. *Mediators Inflamm.* 2006: 1-7.
 66. Lo, H.C., Lin, S.C. and Wang, Y.M. (2004) The relationship among serum cytokines, chemokine, nitric oxide, and leptin in children with type 1 diabetes mellitus. *Clin. Biochem.* 37: 666-672.
 67. Ghasemi, A., Zahediasl, S., Azimzadeh, I. and Azizi, F. (2011) Increased serum nitric oxide metabolites in dysglycaemia. *Ann. Hum. Biol.* 38: 577-582.
 68. Hermenegildo, C., Medina, P., Peiro, M., Segarra, G., Vila, J.M., Ortega, J. and Lluch, S. (2002) Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in hyperthyroid patients. *J. Clin. Endocrinol. Metab.* 87: 5636-5640.
 69. Ueyama, J., Kondo, T., Imai, R., Kimata, A., Yamamoto, K., Suzuli, K., Inoue, T., Ito, Y., Miyamoto, K., Hasegawa, T. and Hamajima, N. (2008) Association of serum NO_x level with clustering of metabolic syndrome components in middle-aged and elderly general populations in Japan. *Environ. Health Prev. Med.* 13: 36-42.
 70. Kagota, S., Yamaguchi, Y., Tanaka, N., Kubota, Y., Kobayashi, K., Nejime, N., Nakamura, K., Kunitomo, M. and Shinozuka, K. (2006) Disturbances in nitric oxide/cyclic guanosine monophosphate system in SHR/NDmcr-cp rats, a model of metabolic syndrome. *Life Sci.* 78: 1187-1196.
 71. Ghasemi, A., Zahediasl, S. and Azizi, F. (2010) Nitric oxide and clustering of metabolic syndrome components in pediatrics. *Eur. J. Epidemiol.* 25: 45-53.
 72. Higashino, H., Miya, H., Mukai, H. and Miya, Y. (2007) Serum nitric oxide metabolite (NO_x) levels in hypertensive patients at rest: a comparison of age, gender, blood pressure and complications using normotensive controls. *Clin. Exp. Pharmacol. Physiol.* 34: 725-731.
 73. Lyamina, N.P., Dolotovskaya, P.V., Lyamina, S.V., Malyshev, I.Y. and Manukhina, E.B. (2003) Nitric oxide production and intensity of free radical processes in young men with high normal and hypertensive blood pressure. *Med. Sci. Monit.* 9: CR304-310.
 74. Ghasemi, A., Zahediasl, S., Syedmoradi, L. and Azizi, F. (2011) Association between serum nitric oxide metabolites and hypertension in a general population. *Int. Angiol.* 30:380-7.
 75. Winlaw, D.S., Smythe, G.A., Keogh, A.M., Schyvens, C.G., Spratt, P.M. and Macdonald, P.S. (1994) Increased nitric oxide production in heart failure. *Lancet* 344: 373-374.
 76. Olszanecka-Glinianowicz, M., Zahorska-Markiewicz, B., Janowska, J. and Zurakowski, A. (2004) Serum concentrations of nitric oxide, tumor necrosis factor (TNF)-alpha and TNF soluble receptors in women with overweight and obesity. *Metabolism* 53: 1268-1273.
 77. Gruber, H.J., Mayer, C., Mangge, H., Fauler, G., Grandits, N. and Wilders-Truschnig, M. (2008) Obesity reduces the bioavailability of nitric oxide in juveniles. *Int. J. Obes. (Lond.)* 32: 826-831.
 78. Carlstrom, M., Larsen, F.J., Nystrom, T., Hezel, M., Borniquel, S., Weitzberg, E. and Lundberg, J.O. (2010) Dietary inorganic nitrate reverses features of metabolic syndrome in endothelial nitric oxide synthase-deficient mice. *Proc. Natl. Acad. Sci. USA* 107: 17716-17720.
 79. Shimabukuro, M., Ohneda, M., Lee, Y. and Unger, R.H. (1997) Role of nitric oxide in obesity-induced beta cell disease. *J. Clin. Invest.* 100: 290-295.
 80. Perreault, M. and Marette, A. (2001) Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat. Med.* 7: 1138-1143.
 81. Duplain, H., Burcelin, R., Sartori, C., Cook, S., Egli, M., Lepori, M., Vollenweider, P., Pedrazzini, T., Nicod, P., Thorens, B. and Scherrer, U. (2001) Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation* 104: 342-345.
 82. Scherrer, U. and Sartori, C. (2000) Defective nitric oxide synthesis: a link between metabolic insulin resistance, sympathetic over activity and cardiovascular morbidity. *Eur. J. Endocrinol.* 142: 315-323.
 83. Carrillo-Sepulveda, M.A., Ceravolo, G.S., Fortes, Z.B., Carvalho, M.H., Tostes, R.C., Laurindo, F.R., Webb, R.C. and Barreto-Chaves, M.L. (2010) Thyroid hormone stimulates NO production via activation of the PI3K/Akt pathway in vascular myocytes. *Cardiovasc. Res.* 85: 560-570.
 84. Napoli, R., Biondi, B., Guardasole, V., Matarazzo, M., Pardo, F., Angelini, V., Fazio, S. and Sacca, L. (2001) Impact of hyperthyroidism and its correction on vascular reactivity in humans. *Circulation* 104: 3076-3080.
 85. Rodriguez-Arnao, M.D., Rodriguez-Sanchez, A., Rodriguez-Arnao, J., Dulin- Iniguez, E., Cano, J.M. and Munoz-Fernandez, M.A. (2003) Undetectable levels of tumor necrosis factor-alpha, nitric oxide and inadequate expression of inducible nitric oxide synthase in congenital hypothyroidism. *Eur. Cytokine. Netw.* 14: 65-68.
 86. Quesada, A., Sainz, J., Wangenstein, R., Rodriguez-

- Gomez, I., Vargas, F. and Osuna, A. (2002) Nitric oxide synthase activity in hyperthyroid and hypothyroid rats. *Eur. J. Endocrinol.* 147: 117-122.
87. Larsen, F.J., Ekblom, B., Sahlin, K., Lundberg, J.O. and Weitzberg, E. (2006) Effects of dietary nitrate on blood pressure in healthy volunteers. *N. Engl. J. Med.* 355: 2792-2793.
88. Ambrosio, G., Del Pinto, M., Tritto, I., Agnelli, G., Bentivoglio, M., Zuchi, C., Anderson, F.A., Gore, J.M., Lopez-Sendon, J., Wyman, A., Kennelly, B.M. and Fox, K.A. (2010) Chronic nitrate therapy is associated with different presentation and evolution of acute coronary syndromes: insights from 52,693 patients in the Global Registry of Acute Coronary Events. *Eur. Heart J.* 31: 430-438.
89. Bryan, N.S., Calvert, J.W., Elrod, J.W., Gundewar, S., Ji, S.Y. and Lefer, D.J. (2007) Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. *Proc. Natl. Acad. Sci. USA* 104: 19144-19149.
90. L'Hirondel J, L., Avery, A.A. and Addiscott, T. (2006) Dietary nitrate: where is the risk? *Environ. Health Perspect.* 114: A458-459.
91. Ward, M.H., deKok, T.M., Levallois, P., Brender, J., Gulis, G., Nolan, B.T. and VanDerslice, J. (2005) Workgroup report: Drinking-water nitrate and health-recent findings and research needs. *Environ. Health Perspect.* 113: 1607-1614.
92. van Grinsven, H.J., Ward, M.H., Benjamin, N. and de Kok, T.M. (2006) Does the evidence about health risks associated with nitrate ingestion warrant an increase of the nitrate standard for drinking water? *Environ. Health.* 5: 26.
93. Gilchrist, M., Winyard, P.G. and Benjamin, N. (2010) Dietary nitrate--good or bad? *Nitric Oxide* 22: 104-109.
94. Powlson, D.S., Addiscott, T.M., Benjamin, N., Cassman, K.G., de Kok, T.M., van Grinsven, H., L'Hirondel, J.L., Avery, A.A. and van Kessel, C. (2008) When does nitrate become a risk for humans? *J. Environ. Qual.* 37: 291-295.
95. World Health Organization (2008) Guidelines for drinking-water quality. Second addendum to third edition, vol. 1, Recommendations. Geneva: World Health Organization.
96. Cannon, R.O., 3rd, Schechter, A.N., Panza, J.A., Ognibene, F.P., Pease-Fye, M.E., Waclawiw, M.A., Shelhamer, J.H. and Gladwin, M.T. (2001) Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery. *J. Clin. Invest.* 108: 279-287.
97. Rassaf, T., Preik, M., Kleinbongard, P., Lauer, T., Heiss, C., Strauer, B.E., Feelisch, M. and Kelm, M. (2002) Evidence for in vivo transport of bioactive nitric oxide in human plasma. *J. Clin. Invest.* 109: 1241-1248.
98. Schechter, A.N. and Gladwin, M.T. (2003) Hemoglobin and the paracrine and endocrine functions of nitric oxide. *N. Engl. J. Med.* 348: 1483-1485.
99. Elrod, J.W., Calvert, J.W., Gundewar, S., Bryan, N.S. and Lefer, D.J. (2008) Nitric oxide promotes distant organ protection: evidence for an endocrine role of nitric oxide. *Proc. Natl. Acad. Sci. USA* 105: 11430-11435.
100. Grau, M., Hendgen-Cotta, U.B., Brouzos, P., Drexhage, C., Rassaf, T., Lauer, T., Dejam, A., Kelm, M. and Kleinbongard, P. (2007) Recent methodological advances in the analysis of nitrite in the human circulation: nitrite as a biochemical parameter of the L-arginine/NO pathway. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 851: 106-123.
101. Gladwin, M.T., Raat, N.J., Shiva, S., Dezfulian, C., Hogg, N., Kim-Shapiro, D.B. and Patel, R.P. (2006) Nitrite as a vascular endocrine nitric oxide reservoir that contributes to hypoxic signaling, cytoprotection, and vasodilation. *Am. J. Physiol. Heart Circ. Physiol.* 291: H2026-2035.
102. Hord, N.G., Tang, Y. and Bryan, N.S. (2009) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am. J. Clin. Nutr.* 90: 1-10.
103. Stamler, J.S., Jaraki, O., Osborne, J., Simon, D.I., Keaney, J., Vita, J., Singel, D., Valeri, C.R. and Loscalzo, J. (1992) Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. *Proc. Natl. Acad. Sci. USA* 89: 7674-7677.
104. Stamler, J.S., Simon, D.I., Osborne, J.A., Mullins, M.E., Jaraki, O., Michel, T., Singel, D.J. and Loscalzo, J. (1992) S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. *Proc. Natl. Acad. Sci. USA* 89: 444-448.
105. Scharfstein, J.S., Keaney, J.F., Jr., Slivka, A., Welch, G.N., Vita, J.A., Stamler, J.S. and Loscalzo, J. (1994) In vivo transfer of nitric oxide between a plasma protein-bound reservoir and low molecular weight thiols. *J. Clin. Invest.* 94: 1432-1439.
106. Troncy, E., Francoeur, M., Salazkin, I., Yang, F., Charbonneau, M., Leclerc, G., Vinay, P. and Blaise, G. (1997) Extra-pulmonary effects of inhaled nitric oxide in swine with and without phenylephrine. *Br. J. Anaesth.* 79: 631-640.
107. Muller, B., Kleschyov, A.L., Alencar, J.L., Vanin, A. and Stoclet, J.C. (2002) Nitric oxide transport and storage in the cardiovascular system. *Ann. NY. Acad. Sci.* 962: 131-139.
108. Kevil, C.G. and Lefer, D.J. (2011) Review focus on inorganic nitrite and nitrate in cardiovascular health and disease. *Cardiovasc. Res.* 89: 489-491.