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# REGULATION OF NITRIC OXIDE SYNTHESIS BY DIETARY FACTORS

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■ **Abstract** Nitric oxide (NO) is synthesized from L-arginine by NO synthase (NOS). As an endothelium-derived relaxing factor, a mediator of immune responses, a neurotransmitter, a cytotoxic free radical, and a signaling molecule, NO plays crucial roles in virtually every cellular and organ function in the body. The discovery of NO synthesis has unified traditionally diverse research areas in nutrition, physiology, immunology, pathology, and neuroscience. Increasing evidence over the past decade shows that many dietary factors, including protein, amino acids, glucose, fructose, cholesterol, fatty acids, vitamins, minerals, phytoestrogens, ethanol, and polyphenols, are either beneficial to health or contribute to the pathogenesis of chronic diseases partially through modulation of NO production by inducible NOS or constitutive NOS. Although most published studies have focused on only a single nutrient and have generated new and exciting knowledge, future studies are necessary to investigate the interactions of dietary factors on NO synthesis and to define the underlying molecular mechanisms.

## CONTENTS

## INTRODUCTION

Animal and human diets contain a complex mixture of nutrients and factors that are beneficial to health. An excess or deficiency of dietary nutrients causes a wide array of chronic and life-threatening diseases. For example, high dietary intake of fructose or saturated fat results in insulin resistance and cardiovascular abnormalities (125). Likewise, a dietary deficiency of protein, vitamin A, or iron impairs immune function and increases the susceptibility of the host to infection (128). Furthermore, dietary factors are now considered to be the principal environmental risk determinants for colorectal cancer (111). Whereas diet-associated diseases have been explained in nutrition textbooks by altered metabolic processes, a unifying mediator of these disorders was not identified until the discovery of the arginine-dependent synthesis of nitric oxide (NO) in 1988.

NO is one of the most widespread signaling molecules and participates in virtually every cellular and organ function in the body. For example, physiological levels of NO produced by endothelial cells (EC) are essential for regulating the relaxation and proliferation of vascular smooth muscle cells (VSMC), leukocyte adhesion, platelet aggregation, angiogenesis, and thrombosis (68). In addition, NO produced by neurons serves as a neurotransmitter, and NO produced by activated macrophages is an important mediator of the immune response (14). However, as an oxidant and as an inhibitor of the enzymes containing an iron-sulfur center, excess production of NO is detrimental to tissues and to cardiovascular function (14, 68). Increasing evidence over the past 10 years shows that many dietary factors affect health and contribute to the pathogenesis of chronic diseases partly through modulation of NO synthesis. The major objective of this article is to review the recent advances in this rapidly growing area of research.

## NO SYNTHESIS IN MAMMALIAN CELLS

### Isoforms of NOS

NO is synthesized from L-arginine and $O_2$ by NO synthase (NOS) (EC 1.14.13.39) in almost all mammalian cells (3). Three distinct isoforms of NOS (nNOS, iNOS,

and eNOS) have been identified, which are encoded by different genes and differ in molecular, catalytic, and immunological properties, cellular distribution, regulation of activity, and sensitivity to inhibitors. nNOS (also known as type I, NOS-I, and NOS-1) was first identified as constitutive in neuronal tissue. iNOS (also known as type II, NOS-II, and NOS-2) was originally identified as being inducible by inflammatory cytokines and lipopolysaccharide (LPS) in macrophages and hepatocytes. eNOS (also known as type III, NOS-III, and NOS-3) was originally identified as constitutive in vascular EC. nNOS and eNOS are collectively termed constitutive NOS (cNOS). However, it is now clear that all three NOS isoforms can be induced by different, appropriate stimuli through transcriptional and posttranscriptional mechanisms and can be constitutively expressed in some tissues or cells (3). In addition, all three isoforms can be found in the cytosol or particulate fractions of cells, or both.

The known NOS enzymes are dimers in their active states, and two NOS monomers are tightly associated with two calmodulin molecules (3). All NOS isoforms contain the relatively tightly bound cofactors (6R)-5,6,7,8-tetrahydrobiopterin (BH4), FAD, FMN, and heme (iron protoporphyrin IX), and also require NADPH and calmodulin for enzymatic activity. NOS is the most recent addition to a group of only four other enzymes (phenylalanine hydroxylase, tyrosine hydroxylase, tryptophan hydroxylase, and alkylglycerol monooxygenase) that are known to utilize a biopterin cofactor (73). Arginine, BH4, and heme promote and stabilize the active dimeric form of all isoforms of NOS. For human, rodent, bovine, and porcine cells and tissues, $Ca^{2+}$ is required for cNOS, but not iNOS, activity. However, such a distinction is less clear-cut for guinea-pig iNOS (126). When arginine or BH4 is deficient, superoxide is generated by NOS, indicating an important role of NOS in cell oxidative stress (89).

## NO Synthesis in Mammalian Cells and the Whole Body

A quantitatively small amount of NO is produced by nNOS or eNOS in mammalian cells, whereas large amounts of NO are generated by iNOS in almost all cell types stimulated by inflammatory cytokines and LPS. For example, the rate of NO production by LPS-activated RAW 264.7 macrophages is 3.3 nmol/$10^6$ cells/h (90), which is $\sim$14 times that by unstimulated bovine venular EC (0.24 nmol/$10^6$ cells/h) (151). Similarly, in vivo systemic production of NO, as assessed by urinary excretion of nitrate (the major stable oxidation end-product of NO) is low in healthy young rats (e.g., 15 $\mu$mol/kg body wt/24 h) fed a 20% casein diet but can increase up to 15-fold in response to immunological challenge (150). In addition, NO synthesis accounts for only 1.2% of the whole body arginine flux in healthy men (18). The quantitatively low rate of NO synthesis in mammalian cells and the whole body should not negate an important role of NO in biology. Rather, because deficiencies or excesses of NO production lead to dysfunctions of numerous and diverse organs and systems (14, 68), understanding the precise mechanisms for dietary regulation of NO synthesis is crucial to the health and survival of animals and humans.

## EFFECTS OF DIETARY FACTORS ON CONSTITUTIVE NO SYNTHESIS

### Protein and Amino Acids

There are few published studies on the effect of dietary protein on constitutive NO production. Feeding a 5% casein diet to young rats resulted in 18% and 52% decreases, respectively, in plasma arginine concentration and whole body constitutive NO production, compared with rats fed a 20% casein diet (150). Thus, substrate availability alone does not fully explain the impaired NO synthesis in protein-deficient rats. One of the underlying mechanisms has been identified to be a decrease in tissue cNOS expression (150). Similarly, feeding a low protein (6%) diet to adult rats decreased both systemic and renal NO production, compared with a high protein (50%) diet (138). These findings help explain the clinical observation that dietary protein deficiency results in cardiovascular abnormalities (148).

Studies in vitro have shown that increasing extracellular arginine concentrations from 0.1 to 10 mM dose-dependently increases NO production by cultured EC in the presence of physiological glutamine concentration (0.5–0.6 mM) (5). Increasing plasma arginine concentrations also increases in vivo constitutive NO synthesis by eNOS in young mammals (152, 153). For example, feeding a 1% arginine diet to young rats increased plasma arginine concentration and in vivo constitutive NO production by 105% and 73%, respectively, compared with rats fed an arginine-free diet (150). However, such an effect of arginine was not observed in healthy adult men fed an arginine-free diet for 6 days (19), reflecting an adequate endogenous synthesis of arginine for maintaining short-term NO production. Importantly, arginine supplementation has been shown to improve NO-dependent endothelial relaxation in patients with major cardiovascular risk factors (hypercholesterolemia, smoking, hypertension, diabetes, obesity, insulin resistance, and aging) and with many common cardiovascular disorders (coronary and peripheral artery disease, ischemia/reperfusion injury, heart failure, and erectile dysfunction) (89, 152). Remarkably, vascular endothelial dysfunction occurred in a patient with a natural deficiency of arginine (plasma arginine $= 21\ \mu$M) owing to a defect in the basic amino acid transporter (69). Thus, compelling evidence exists to show that endothelial NO synthesis can be regulated by changes in extracellular or intracellular arginine concentrations.

Interestingly, the Km value of eNOS for arginine is only 2.9 $\mu$M (109), which is much lower than the intracellular arginine concentration (0.5–3 mM) in almost all cell types studied (28, 82, 154) except hepatocytes ($<$0.04 mM) (120). This "arginine paradox" for NO synthesis has been explained by a number of theories, which include colocalization of the arginine transporter, CAT-1, and eNOS in membrane-associated caveolae, insulin release, intracellular compartmentation of arginine, promotion of NOS dimerization, and competitive inhibition of eNOS by endogenous inhibitors [e.g., asymmetric dimethylarginine (ADMA)] (89). However, none of these theories satisfactorily explain the "arginine paradox". For example, an increase in arginine transport by EC overexpressing arginase I and II

is not associated with a concomitant increase in NO production (82). In addition, given the high arginine concentration in many cells, including EC, it is unlikely that compartmental arginine concentration at the site of eNOS is as low as 3 $\mu$M. Furthermore, the arginine concentration required for promoting NOS dimerization and stability is <0.1 mM (3). Finally, the plasma concentration of ADMA is very low ($\sim$0.1 $\mu$M) in healthy subjects (143) compared with that of arginine (90–250 $\mu$M) (154) and is insufficient to block endothelial NO production in vivo.

Glutamine is an important regulator of endothelial NO synthesis. Vane's group reported in 1990 that glutamine markedly decreased NO production in porcine EC (57) and intact blood vessels (134). Subsequent work has shown that increasing extracellular L-glutamine concentrations from 0 to 2 mM dose-dependently reduces NO synthesis by bovine and porcine EC under both basal and stimulated conditions (5, 91). In addition, glutamine inhibits NO-dependent cerebral neurogenic vasodilation (81). There is also in vivo evidence showing that dietary glutamine supplementation inhibits systemic NO production in rats (62). Neither glutamine nor its metabolites (e.g., ammonia, glutamate, alanine, and aspartate) have an effect on eNOS expression or intracellular concentrations of NOS substrates and cofactors, including BH4, $Ca^{2+}$, and NADPH (151). Using cultured bovine venular EC, we have recently discovered that glutamine metabolism to glucosamine-6-phosphate is necessary for glutamine inhibition of endothelial NO synthesis (151).

Glutamate plays an important role in neurological function. Glutamate binds with the excitotoxic N-methyl-D-aspartic acid receptor, resulting in an increase in the influx of $Ca^{2+}$ and its intracellular concentration and consequently activation of nNOS for NO synthesis (6). The glutamate-induced increase in NO synthesis in brain and other nervous tissues provides a metabolic basis to explain the long-standing glutamate neurotoxicity, and has important implications for dietary glutamate supplementation to patients with spinal cord or brain injury.

Lysine shares the same transport system ($y^+$) with arginine for entry into cells, and thus an increase in extracellular lysine concentration would be expected to decrease the intracellular availability of arginine for constitutive NO synthesis. This proposition is supported by findings that the stretch-induced increase in NO production by rat pulmonary arteries is inhibited by a high concentration of extracellular lysine (64). This effect of lysine is likely more pronounced when plasma arginine concentration is relatively low, and helps explain, in part, the classical arginine-lysine antagonism in nutrition.

Homocysteine is an independent risk factor of cardiovascular disease and thus has attracted considerable attention in recent years (25). This sulfur-amino acid is synthesized from methionine primarily via the transsulfuration pathway and is converted to methionine by methionine synthase, which requires 5-methyltetrahydrofolate and methylcobalamin (vitamin B12) as cofactors. There is direct evidence to show that increasing extracellular concentrations of homocysteine decreases NO synthesis by EC (157) and platelets (99), thereby impairing endothelium-dependent relaxation. Similarly, dietary methionine loading or hyperhomocysteinemia causes endothelial dysfunction, which can be alleviated by agents (e.g., folic acid and

vitamin B12) that reduce plasma homocysteine concentration (25). Little is known about the mechanism for the inhibition of endothelial NO synthesis by homocysteine. Interestingly, a recent study reported that culture of bovine aortic EC with either 0.01-1 mM DL-homocysteine or 0.03-1 mM L-methionine increased ADMA accumulation in the culture medium in a dose- and time-dependent manner (130a). This effect of homocysteine and methionine was associated with a decreased activity of dimethylarginine dimethylaminohydrolase, the enzyme that degrades ADMA. There was also evidence showing that homocysteine dose-dependently decreased the activity of recombinant human dimethylarginine dimethylaminohydrolase in a cell-free system (130a). Although intracellular ADMA concentrations were not determined in this study, these results suggest that methionine is metabolized to homocysteine in EC and that homocysteine decreases endothelial NO synthesis by elevating ADMA production owing to an inhibition of its degradation. It is of nutritional and physiological importance to determine whether EC contain the complete transsulfuration pathway, or a truncated pathway (via methionine adenosyltransferase, S-adenosylmethionine-dependent methyltransferase, and S-adenosylhomocysteine hydrolase), for the production of homocysteine from methionine.

Citrulline is an effective precursor for arginine synthesis in virtually all animal cells (154). Thus, increasing extracellular concentration of citrulline can support constitutive NO synthesis in many cell types, including EC, VSMC, and neurons (96). Studies in vivo have also demonstrated that dietary supplementation of citrulline reduces blood pressure in salt-sensitive hypertensive rats (27). As a neutral amino acid, citrulline does not compete with basic amino acids for transport by cells, its conversion into arginine consumes ammonia in the form of aspartate (154), and its administration does not require equimolar HCl (27). Thus, enteral or parenteral provision of citrulline may be particularly useful for patients with elevated ammonia concentrations, impaired arginine transport, or enhanced intestinal arginine catabolism.



## Carbohydrates

Glucose metabolism via the pentose cycle is the major source of NADPH for NO synthesis (151). Thus, in intact cells, NO synthesis is glucose-dependent, and a deficiency of glucose results in reduced synthesis of NO by EC and many other cell types. However, elevated glucose concentrations may be detrimental to cells and tissues, particularly in diabetic patients. For example, in vitro studies have shown that increasing extracellular glucose concentrations from 5 to 30 mM dose-dependently decreases NO production in cultured EC, rat mesangial cells, and VSMC (21, 139). Likewise, acute hyperglycemia impairs NO-mediated endothelial function and induces insulin insensitivity in healthy subjects in vivo (149). Similar vascular dysfunction occurs in diabetic patients with chronic hyperglycemia owing to poor metabolic control (51). The mechanisms for the inhibitory effect of hyperglycemia on NO synthesis are not known at present.

Glucose metabolism to glucosamine may be necessary for glucose inhibition of endothelial NO synthesis (151), and advanced glycosylation end products may also inhibit cNOS expression in EC (21). Interestingly, a recent study reported that culture of bovine aortic EC with 30 mM glucose or 5-10 mM glucosamine increased *O*-linked *N*-acetylglucosamine modification of eNOS protein and decreased eNOS activity, as compared with 5 mM glucose (38a). Remarkably, antisense inhibition of glutamine:fructose-6-phosphate amidotransferase [also known as glutamine:fructose-6-phosphate transaminase (EC 2.6.1.16)] reversed the change in eNOS posttranslational modification and the reduction of eNOS activity brought about by hyperglycemia (38a). Because glutamine:fructose-6-phosphate amidotransferase is present for de novo synthesis of UDP-*N*-acetylglucosamine in EC and this pathway is modulated by hyperglycemia (151a), prevention of hyperglycemia-induced activation of the hexosamine synthetic pathway may help prevent the deficiency of endothelial NO synthesis as well as the development and progression of vascular dysfunction in diabetes mellitus.

Whereas most published studies have found that hyperglycemia inhibits NO synthesis in EC from large vessels, a few studies have reported the opposite for microvascular EC. For example, elevating glucose concentrations increased optic nerve head circulation via an increase in NO synthesis (78). In addition, hyperglycemia increased NO synthesis in capillary EC isolated from rat islets (131) and in glomerular EC (72). This stimulatory effect of glucose on NO synthesis may play a role in the pathogenesis of retinal degeneration, $\beta$-cell dysfunction, and nephropathy in diabetes.

Increasing dietary intake of fructose has long been known to impair vascular relaxation, cause hypertension, and induce insulin resistance in the rat model (135). These findings imply, but do not necessarily indicate, an inhibition of endothelial NO synthesis by fructose. Interestingly, administration of BH4 prevented the fructose-induced vascular dysfunction and insulin resistance (125), suggesting a deficiency of cellular BH4. However, this view should be supported by a direct measurement of BH4 in EC.

## Saturated Fats, Cholesterol, and Low-Density Lipoprotein

Excess consumption of dietary saturated fat increases plasma concentrations of cholesterol and low-density lipoprotein (LDL), established risk factors for cardiovascular disease (15). For this reason, much research has been directed at studying the role of saturated fats and fatty acids on endothelial NO production. For example, feeding a diet containing high saturated fat impaired endothelium-dependent relaxation in pregnant rats and their offspring (45), suggesting a decrease in NO production by EC. Similarly, in both apoE-deficient mice (39) and patients with type II diabetes (40) fed a Western-type high fat diet, there was a reduction in endothelial NO generation and endothelium-dependent relaxation. Furthermore, elevating plasma concentrations of triglycerides and saturated fatty acids impaired endothelial NO production and insulin-mediated vasodilation in vivo (159). At

present, little is known about the mechanisms for the inhibitory effect of saturated fats on NO synthesis by EC. However, an increase in acylation of eNOS by myristate (C14:0) and palmitate (C16:0), which accelerates the association of eNOS with caveolae and therefore inhibits eNOS activity (3), may contribute to decreased endothelial NO synthesis.

Much evidence shows that endothelial NO synthesis is impaired in hypercholesterolemic patients, resulting in endothelial dysfunction (89). Thus, there is an inverse relationship between very low density lipoprotein (VLDL) or cholesterol and plasma concentrations of nitrite plus nitrate (136). The mechanisms for the inhibitory effect of LDL and hypercholesterolemia on NO synthesis are complex. First, oxidized LDL inhibits agonist-stimulated arginine transport by EC and eNOS expression (both mRNA and protein levels), thereby directly inhibiting NO production (58). Second, oxidized LDL upregulates the synthesis of ADMA in EC and plasma concentrations of ADMA are elevated in hypercholesterolemic subjects (12). Third, increased uptake of cholesterol by EC results in increased abundance of the membrane-bound structural protein caveolin-1 and impairment of NO production through stabilization of the inhibitory heterocomplex between caveolin-1 and eNOS (41). Fourth, BH4 availability in EC may be reduced in hypercholesterolemic patients, as BH4 administration prevents endothelial dysfunction in these patients (129). Finally, oxidized LDL causes an intracellular dislocation of eNOS in EC, including translocation from the plasma membrane and disintegration of the Golgi, thereby inhibiting NO production (101). Thus, through a reduction in plasma concentrations of LDL and VLDL, inhibition of cholesterol synthesis by statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) enhances NO production in EC and alleviates endothelial dysfunction in hypercholesterolemic patients (58).



## Unsaturated Fatty Acids and Sphingolipids

Epidemiological studies have shown that the consumption of fish oils, which are rich in $\omega$-3 fatty acids, is associated with reduced morbidity and mortality from coronary heart disease (15). Available evidence shows that polyunsaturated fatty acids regulate the function of the vascular endothelium partially through alterations in NO synthesis. For example, eicosapentaenoic acid (C20:5$\omega$3) has been shown to increase NO synthesis by EC and improve endothelium-dependent relaxation in vitro through multiple mechanisms, including an increase in intracellular $Ca^{2+}$ concentration and translocation of eNOS (104, 105). Similarly, treatment of isolated rat aortic rings with docosahexaenoic acid (C22:6$\omega$3) enhances NO synthesis in EC and relaxation of VSMC (80). These findings provide a biochemical basis for clinical observations that dietary supplementation of fish oil, eicosapentaenoic acid, or docosahexaenoic acid markedly increases urinary excretion of nitrate (56) and has a beneficial effect on cardiovascular function (15).

Addition of linoleic acid (C18:2$\omega$6) to isolated rat aorta has no vasorelaxant effect, suggesting unaltered NO production in EC (61). Similarly, the 13-hydroxy metabolites of linoleic acid (13-hydroxylinoleic and 13-hydroperoxylinoleic acids)

do not affect NO production in canine arteries (36). Interestingly, diets rich in linoleic acid reduce blood pressure and prevent coronary arterial disease (15). These apparent discrepancies between in vitro and in vivo observations may be explained by the fact that linoleic acid is actively used for in vivo synthesis of trilinolein, which induces NO-dependent vasodilation (61). Note that oxidized linoleic acid increases NO production in EC through an upregulation of eNOS expression (113), which may serve as a compensatory mechanism for endothelial oxidative injury during the early stage of hypercholesterolemia.

In contrast to $\omega$-3 and $\omega$-6 polyunsaturated fatty acids, oleic acid (C18:1$\omega$9) inhibits endothelial NO synthesis by decreasing eNOS activity, resulting in impaired endothelium-dependent vasorelaxation (34). Likewise, an elevation of rat portal concentration of oleic acid owing to its intravenous infusion causes an increase in blood pressure (49). These results suggest that increasing plasma concentrations of oleic acid or other $\omega$-9 unsaturated fatty acids may contribute to the pathogenesis of endothelial dysfunction.

Sphingolipids are natural metabolites of lipids and are inhibitors of carcinogenesis (92). Interestingly, sphingolipids can regulate NO synthesis in a cell-specific manner. For example, sphingosine-1-phosphate stimulates NO synthesis in EC through the $G_i$/phosphoinositide-3-kinase pathway and eNOS phosphorylation (95). Ceramide also stimulates NO production in bovine aortic EC through eNOS translocation and activation (67). In contrast, sphingosine inhibits nNOS activity in differentiated cerebellar granule cells through interfering with the calmodulin-dependent activation of eNOS (142).

## Vitamins

Vitamin C (ascorbate) (65), vitamin E ($\alpha$-tocopherol) (74), folic acid (130), and vitamin A (all-trans retinoic acid) (2) all increase NO synthesis in EC, consistent with their role as antiatherosclerotic agents. In addition, vitamin C antagonizes the inhibition of endothelial NO synthesis by hyperglycemia (9), whereas both vitamin A and vitamin E promote NO production in neuronal cells (108). Thus, dietary supplementation of vitamin E and 5-methyltetrahydrofolate (the active form of folic acid) restores endothelial NO synthesis and improves endothelium-dependent relaxation in hypercholesterolemic patients (15). As expected from their diverse chemical properties, vitamins promote endothelial NO synthesis through different mechanisms. For example, vitamin C increases cellular BH4 availability in EC by stabilizing BH4 (65), and vitamin E stimulates agonist-induced eNOS activation (74). Also, 5-methyltetrahydrofolate directly enhances eNOS activity in both recombinant enzyme and cultured EC (130). Interestingly, vitamin A reduces the cellular concentration of ADMA in EC by inhibiting the expression of dimethylarginine dimethylaminohydrolase (2), while increasing nNOS expression (108).

## Minerals

The chemical properties of NOS exemplify the crucial roles of minerals in NO synthesis. $Ca^{2+}$ is required for eNOS and nNOS activity, and thus, increasing

extracellular or intracellular $Ca^{2+}$ concentrations stimulate NO production by EC (5, 91). This aids in explaining the finding that a high calcium diet attenuates the development of hypertension in the spontaneously hypertensive rat (86). The iron-containing heme is also an essential component of NOS, and thus, iron availability can modulate NO synthesis. Indeed, an iron deficiency has been reported to reduce nNOS (133) and ileal NOS (47) activity in rats. In addition, zinc is bound to all isoforms of NOS (3) and dimethylargininase (13), thereby modulating NOS activity. At elevated concentrations zinc inhibits nNOS and eNOS activity (107), as well as NO production by brain tissues (103), rat VSMC (1), and bovine pulmonary artery EC (1).

Several other minerals have been reported to affect constitutive NO synthesis. For example, an elevated concentration of copper inhibits nNOS activity and NO production in glial cells (29). In contrast, increasing extracellular concentrations of manganese stimulates NO synthesis in murine astrocytes (127). Similarly, administration of manganese or lead increases the expression and activity of nNOS in various regions of the rat brain (114), suggesting that an increased exposure to toxic levels of these elements may interfere with $Ca^{2+}$-mediated NO synthesis and lead to neuronal dysfunction. In addition, consistent with its well-known vasodilator effect, increasing plasma magnesium concentrations (via infusion of $MgSO_4$) in healthy humans dose-dependently enhances endothelial NO production and blood flow in the forearm vasculature (35). Thus, a deficiency of magnesium (commonly observed in patients undergoing cardiac operations) results in decreased NO synthesis by EC, but magnesium supplementation restores normal endothelium-dependent relaxation (106).



## Glucosamine

Glucosamine is a natural metabolite synthesized from fructose-6-phosphate (a glucose metabolite) and glutamine. Glucosamine is taken up by EC via glucose transporters for conversion to glucosamine-6-phosphate, a competitive inhibitor of glucose-6-phosphate dehydrogenase (70). Interestingly, Holmang et al. reported that infusion of glucosamine to rats markedly decreased blood flow in hindlimb femoral muscles (60), implying an inhibition of endothelial NO synthesis by glucosamine. Using cultured EC, we have recently shown that glucosamine inhibits NO production in a concentration-dependent manner by decreasing cellular free NADPH availability owing to an inhibition of pentose cycle activity (151). These novel findings may have important implications for endothelial insulin resistance and cardiovascular complications in diabetic and obese subjects.

## Phytoestrogen

Phytoestrogens are a group of dietary substances with structures similar to estradiol, a steroid hormone that increases eNOS activity and NO production by EC (83). Thus, phytoestrogens enhance NO synthesis and endothelium-dependent relaxation in isolated rat aorta (158) or isolated pulmonary arteries from chronically

hypoxic rats (71). Similarly, global myocardial ischemia-reperfusion injury is attenuated in hearts from female rats fed a phytoestrogen-supplemented diet (156). In support of the in vitro findings, the infusion of genistein to healthy humans dose-dependently induced NO-dependent dilation of the forearm vasculature, as did 17-$\beta$-estradiol (145). These findings explain the beneficial effect of phytoestrogen-rich foods, such as soybeans and their products, on the cardiovascular system (83).

## Ethanol and Polyphenols

Epidemiological studies suggest that moderate consumption of red wine is associated with a decrease in the risk of cardiovascular disease. For example, people living in certain regions of France, where red wine is customarily consumed during meals, have a low mortality from coronary heart disease, despite a high dietary intake of saturated fat (155). Thus, there has been considerable interest in the past decade in resolving this so-called "French paradox". Because red wine contains both ethanol and polyphenolic compounds, numerous studies have been conducted to determine their effect on NO production.

Acute exposure of the aorta (79), neurons (23), and hepatic stellate cells (117) to high concentrations of ethanol inhibits constitutive NO synthesis. In contrast, prolonged exposure (24–48 h) of cerebral pial cells to high concentrations of ethanol increases NO production (121). Unfortunately the nutritional and physiological significance of these in vitro findings are not clear because the concentrations of ethanol used (up to 150 mM) are generally much greater than those found in humans with modest consumption of red wine (155). For example, modest consumption of ethanol increases systemic NO production in humans (88), which is consistent with the recent report that ethanol, at low concentrations (e.g., 2–17 mM), stimulates NO production by EC (48, 141). Remarkably, all studies have consistently demonstrated that chronic consumption of relatively high amounts of ethanol decreases NO-dependent endothelial relaxation and causes cardiovascular injury in rats (e.g., 54, 131). Interestingly, provision of BH4 restores endothelial function in rats with chronic alcohol consumption (131), suggesting a decreased availability of BH4 in EC. This view, however, should be supported by a direct measurement of BH4.

Early in 1993 Fitzpatrick et al. (42) first reported that red wine, extracts from grape juices and skin, or quercitin and tannic acid (compounds known to be present in grape skins) produced endothelium-dependent relaxation through increased NO synthesis. Subsequent work has shown that some polyphenolic compounds (e.g., resveratrol, flavonoids, and leucocyanidol), which are abundant in red wine, also increase NO synthesis by EC (4, 66) and platelets (43). Thus, administration of resveratrol to rats increased NO synthesis and reduced ischemia-reperfusion injury in both kidneys (46) and heart (66). There is also in vivo evidence showing that dietary supplementation of purple grape juice or red wine phenols to humans increases NO production by platelets, reduces platelet aggregation, and decreases blood pressure (38). In addition, modest consumption of red wine increases whole

body NO production in healthy humans independent of its alcohol content (88). At present, little is known about the mechanisms responsible for the stimulatory effect of ethanol or polyphenols on constitutive NO synthesis.

## EFFECTS OF DIETARY FACTORS ON INDUCIBLE NO SYNTHESIS

### Protein and Amino Acids

Increasing extracellular arginine or citrulline concentrations from 50 to 400 $\mu$M dose-dependently increases NO synthesis in activated macrophages (75, 96), indicating an important role for arginine availability in inducible NO production. Consistent with this in vitro finding, feeding an arginine- or protein-deficient diet to young rats reduces plasma arginine concentrations and prevents maximal inducible NO synthesis (150). On the basis of the finding that a deficiency of dietary protein, but not dietary arginine, decreases iNOS activity in rat tissues (150), it is likely that a deficiency of a mixture of essential plus nonessential amino acids is necessary to reduce tissue iNOS expression. In support of these findings, dietary protein-calorie malnutrition (119) or an arginine deficiency (16) reduces inducible NO synthesis in wounds. Whereas the arginine-dependent NO synthesis plays an important role in immune responses, an increase in arginine supply for inducible NO production would "fuel the fire" and thus be detrimental under conditions such as hypotension in septic shock, inflammatory bowel disease, renal mesangial injury and fibrosis, and pancreatic $\beta$-cell destruction by activated immune cells (153).

In contrast to constitutive NO generation, glutamine is required for inducible NO synthesis by lipopolysaccharide (LPS)- and cytokine-activated macrophages (11). Maximal inducible NO synthesis by these cells is obtained at 1 mM glutamine, which is greater than the physiological plasma concentration of glutamine in healthy subjects. In addition, our recent study has shown that glutamine is required for the expression of iNOS protein in LPS-activated RAW 264.7 macrophages, with maximal levels of iNOS protein obtained at 2 mM glutamine (C. J. Meininger & G. Wu, unpublished data). Thus, lowered levels of circulating glutamine, which occur often under catabolic conditions such as infection, injury, sepsis, trauma, and cancer, result in suboptimal inducible NO synthesis, which then contributes to impaired host responses to immunologic challenge.

Other amino acids also play a role in regulating inducible NO synthesis. For example, glutamate has been implicated in iNOS expression in ischemic brain (17). Also, increasing extracellular lysine concentrations reduces intracellular arginine concentrations and NO synthesis by activated macrophages (28), further supporting the view that intracellular arginine concentrations are critical for inducible NO production (96). In addition, taurine (a $\beta$-amino acid present only in animal products) and taurine chloramine inhibit iNOS expression and inducible NO synthesis in various cell types, including hepatocytes (115), macrophages (8), and glial

cells (84), suggesting that taurine supplementation may protect the host against oxidant-induced tissue damage.

## Carbohydrates

Elevated concentrations of glucose (up to 30 mM) inhibit iNOS expression and inducible NO production in LPS- or cytokine-activated macrophages (140), aortic EC (52), VSMC (10), and mesangial cells (112). Likewise, high concentrations of fructose inhibit inducible NO synthesis, and thus dietary fructose feeding is beneficial in suppressing LPS-stimulated NO production in vivo (55). Importantly an exogenous supply of BH4 alleviates the inhibitory effect of hyperglycemia on inducible NO synthesis in mesangial cells (112), suggesting a deficiency of cellular BH4. However, such a putative mechanism for the action of hyperglycemia cannot be firmly established without a direct measurement of intracellular BH4.



## Saturated Fatty Acids, Cholesterol, and Low-Density Lipoprotein

Increasing concentrations of free fatty acids induces iNOS expression and increases inducible NO synthesis in normal rat islets, and to a greater extent in islets from Zucker diabetic fatty rats (123). Ceramide appears to mediate the induction of iNOS expression by long-chain fatty acids (124). Similarly, LDL induces NO synthesis in cultured VSMC (85, 110) and macrophages (94). Interestingly, a recent study with C6 glial cells shows that an increase in cellular concentrations of very long-chain fatty acids (C22:0 and C:26), an inherited metabolic disorder with subsequent manifestation of neuroinflammatory disease in humans, enhances cytokine-induced NO production (77). Consistent with these in vitro studies, feeding a high saturated-fat diet to rats increases iNOS activity in liver (147) and colon (146). Collectively, these findings suggest an important role for saturated fatty acids, cholesterol, and LDL in the pathogenesis of $\beta$-cell destruction or dysfunction and of liver, gastrointestinal, vascular, and neurological diseases.

## Unsaturated Fatty Acids and Sphingolipids

Polyunsaturated fatty acids are involved in inflammation, and thus there have been extensive studies of their roles in inducible NO production. Arachidonic acid ($\omega$-6) and $\omega$-3 polyunsaturated fatty acids (docosahexaenoic acid, eicosapentaenoic acid, and $\alpha$-linolenic acid) inhibit inducible NO synthesis in cytokine-activated macrophages by inhibiting iNOS transcription (32, 76, 102). Polyenoic fatty acids with 22 carbons are more inhibitory than those with 20 carbons, and among 22-carbon fatty acids, those with more double bonds and a double bond in the $\omega$-3 position are more inhibitory (76). In contrast, ceramide and linoleic acid enhance iNOS expression and inducible NO synthesis in C6 glioma cells and macrophages (32). Thus, polyunsaturated fatty acids exert differential effects on inducible NO production. These in vitro studies provide a biochemical basis for the recent finding

that dietary supplementation of fish oil ($\omega$-3) reduced NO production by mouse macrophages in response to ocular *Herpes simplex* type I infection (30); however, they cannot be readily reconciled with the report that dietary fish oil enhanced inducible NO production by rat bronchoalveolar macrophages compared with safflower oil ($\omega$-6) (20). A possible explanation is that the proportion of cellular $\omega$-3 and $\omega$-6 fatty acid concentrations is a major determinant of in vivo NO production by iNOS.

## Vitamins

Consistent with its anti-infective role, vitamin A stimulates NO synthesis by activated macrophages via an increase in iNOS expression (7). Intraperitoneal administration of vitamin A to LPS-treated rats also enhances both iNOS mRNA and protein levels in several organs, including liver, kidney, and spleen (37), which may be accounted for largely by macrophages present in these tissues. In contrast, vitamin A inhibits iNOS gene transcription and inducible NO synthesis in cultured VSMC (59), EC (50), cardiac myocytes (50), and mesangial cells (33). Such diametrically opposed effects of vitamin A on iNOS expression may be related to cell types and their functions. For example, an increase in NO synthesis by iNOS in activated macrophages is crucial for the killing of pathogenic microorganisms and the prevention of platelet adhesion and foam cell formation. On the other hand, a decrease in inducible NO production in EC, cardiac myocytes, and mesangial cells precludes endothelial dysfunction and glomerular inflammatory injury.

1,25-dihydroxyvitamin D-3 (the active form of vitamin D3) enhances iNOS expression and inducible NO production in a human macrophage-like cell line (116). 1,25-dihydroxyvitamin D-3 treatment also increased the killing of *Mycobacterium tuberculosis*, which was inhibited by $N^G$-monomethyl-L-arginine (an inhibitor of NOS) (116), suggesting an important role for the vitamin D–induced NO production in host defense. In contrast, 1,25-dihydroxyvitamin D-3 inhibits iNOS expression in inflammatory cells of the rat brain (macrophages, microglia, and astrocytes) and alleviates the clinical syndromes of experimental allergic encephalomyelitis (44). Remarkably, the effect of 1,25-dihydroxyvitamin D-3 on the central nervous system is region-specific and is most pronounced in the cerebellum and brain stem (44). These results suggest a potentially novel therapeutic role of 1,25-dihydroxyvitamin D-3 in the prevention and treatment of iNOS-associated diseases of the central nervous system.

Other vitamins also regulate inducible NO synthesis. For example, niacin inhibits iNOS expression and inducible NO synthesis in the bleomycin-mouse model of lung fibrosis (53). In addition, many carotenoids are capable of suppressing inducible NO synthesis in human promyelocytic HL-60 cells and mouse macrophages (98). This finding explains the in vivo observation that dietary supplementation of $\beta$-carotene to patients with nonatrophic gastritis decreased iNOS protein expression in gastric antral tissue (87). Furthermore, vitamin K2 inhibits iNOS expression and inducible NO synthesis in VSMC (118), further supporting its antiatherogenic effect.

## Minerals

Through regulation of iNOS expression and enzyme activity, several minerals play an important role in inducible NO synthesis. For example, increasing extracellular concentrations of nonheme iron enhances iNOS protein expression and NO synthesis in cultured proximal tubule cells and macrophages (26). In contrast, iNOS protein levels are markedly increased in both thoracic aorta and kidney of iron-deficient rats, which is associated with increased plasma concentrations and urinary excretion of nitrate in anemic animals (100). Thus, interactions between iron and other molecules are likely critical for the regulation of iNOS expression in vivo.

Consistent with its role as an antiinflammatory nutrient, elevated concentrations of zinc inhibit inducible NO synthesis in LPS-treated aortas and VSMC (1) and iNOS activity in activated macrophages (137). Accordingly, a dietary deficiency of zinc enhances iNOS expression in the rat small intestine in response to IL-1$\alpha$ treatment, which is associated with increased incidence of diarrhea, and dietary supplementation of zinc to zinc-deficient rats attenuates intestinal iNOS expression and prevents diarrhea (31). These results implicate an important role of zinc in modulating vascular, immunological, and intestinal function.

Lead, mercury, cadmium, and copper inhibit, but nickel and cobalt increase, inducible NO synthesis in cytokine-activated murine macrophages primarily by inhibiting iNOS activity and enhancing iNOS expression, respectively (137). Chromium also inhibits iNOS expression in these cells (137). Thus, alterations in NO synthesis may mediate, in part, the cytotoxic action of environmental metal contaminations.

## Phytoestrogens

There is little information about the effect of phytoestrogens on inducible NO synthesis. Using the MCF-7 human breast cancer cell line, Hsu et al. (63) reported that the phytoestrogen biochanin A dose-dependently inhibited iNOS expression, inducible NO synthesis, and cell growth. Whether phytoestrogens may protect against tumorigenesis in vivo (83) through an inhibition of inducible NO production remains to be determined.

## Glucosamine

Using an in vivo rat model of LPS-induced inflammation, we recently found that intravenous administration of D-glucosamine (0.5 mmol/kg body wt) 6 h before, at the time of, and 6 h after intraperitoneal LPS injection (1 mg/kg body wt) decreased whole body NO synthesis by 31% and 48%, respectively, at days 1 and 2 post-LPS administration (90). The glucosamine treatment also markedly decreased iNOS protein levels in spleen, lung, and peritoneal macrophages. When cultured macrophages were treated with LPS to induce iNOS expression, addition of 0.1, 0.5, 1, and 2 mM D-glucosamine decreased NO production by 18%, 38%, 60%, and 89%, respectively (90). Glucosamine had no effect on cellular concentrations of arginine, NADPH, or BH4 but dose-dependently suppressed iNOS protein

expression (90). Interestingly, glucosamine or N-acetylglucosamine (a metabolite of glucosamine) has recently been reported to markedly inhibit iNOS expression and inducible NO synthesis in IL-1$\beta$–stimulated human articular chondrocytes (122). Collectively, these novel findings provide a biochemical basis for the use of glucosamine in preventing and treating NO-mediated chronic inflammatory diseases such as arthritis.

### Ethanol and Polyphenols

High concentrations of ethanol (up to 150 mM) inhibit inducible NO production by cytokine-activated macrophages (22, 24) and C6 glioma cells (93). At lower concentrations ethanol has no effect on iNOS expression in these cells (24, 93). The polyphenols quercetin (0.1 and 0.2 mM) and resveratrol (0.05 and 0.1 mM) markedly suppress iNOS expression and inducible NO production in macrophages (144), indicating a more potent inhibitory effect of the polyphenols than ethanol. There is a synergy between ethanol (20–150 mM) and the polyphenols in the inhibition of the iNOS pathway in vitro (22, 24). In contrast to these in vitro studies, oral administration of red wine polyphenolic compounds (20 mg/kg body wt) to normotensive rats enhances iNOS protein expression within the arterial wall (38). Thus, the in vivo interactions between ethanol and polyphenols are complex depending on cell type and may have important implications for immunological, neurological, and cardiovascular dysfunction.



## PERSPECTIVES AND FUTURE DIRECTIONS

NO is one of the smallest and simplest biologically active molecules with enormous versatility and importance in the body. The arginine-dependent synthesis of NO plays an important role in mediating the beneficial or detrimental effect of dietary factors in a cell- or tissue-specific manner. In view of their diverse chemical properties, dietary factors regulate constitutive and inducible NO production likely through different mechanisms, including changes in intracellular concentrations of NOS substrates and cofactors, as well as alterations in NOS expression and kinetic properties. Although great advances have been made in this rapidly-growing field, there is a paucity of information about the roles of many dietary substances on NO production by mammalian cells and thus, this will remain an active area of future investigations. As arginine plays a crucial role in NO production by all isoforms of NOS, there is also a need to fully understand the regulation of arginine metabolism (synthesis and catabolism) at molecular, cellular, and whole body levels (97) and to explain the long-standing "arginine paradox" for NO generation (89, 152). Furthermore, much work is required to understand the complexity of the role of BH4 in NO synthesis and how the pathways of BH4 metabolism are modulated by dietary factors. Finally, while most published studies of NO synthesis have focused on only a single nutrient, future studies are necessary to investigate the interactions of dietary factors on NO synthesis and to define the underlying molecular mechanisms.

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## LITERATURE CITED

1. Abou-Mohamed G, Papapetropoulos A, Catravas JD, Caldwell RW. 1998. $Zn^{2+}$ inhibits nitric oxide formation in response to lipopolysaccharides: implications in its anti-inflammatory activity. *Eur. J. Pharmacol.* 341:265–72
2. Achan V, Tran C, Maria JS, Birdsey GM, Whitley GSJ, et al. 2000. All trans-retinoic acid increases nitric oxide synthesis by endothelial cells: a role for the induction of dimethylarginine dimethylaminohydrolase. *Circulation* 102 (Suppl. S): 68
3. Alderton WK, Cooper CE, Knowles RG. 2001. Nitric oxide synthases: structure, function and inhibition. *Biochem. J.* 357:593–615
4. Andriambeloson E, Kleschyov AL, Muller B, Beretz A, Stoclet JC, Andriantsitohaina R. 1997. Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. *Br. J. Pharmacol.* 120:1053–58
5. Arnal JF, Munzel T, Venema RC, James NL, Bai CL, et al. 1995. Interactions between L-arginine and L-glutamine change endothelial NO production. *J. Clin. Invest.* 95:2565–72
6. Atlante A, Calissano P, Bobba A, Giannattasio S, Marra E, Passarella S. 2001. Glutamate neurotoxicity, oxidative stress and mitochondria. *FEBS Lett.* 497:1–5
7. Austenaa LMI, Ross AC. 2001. Potentiation of interferon-$\gamma$-stimulated nitric oxide production by retinoic acid in RAW 264.7 cells. *J. Leuk. Biol.* 70:121–29
8. Barua M, Liu Y, Quinn MR. 2001. Taurine chloramine inhibits inducible nitric oxide synthase and TNF-$\alpha$ gene expression in activated alveolar macrophages. *J. Immunol.* 167:2275–81
9. Beckman JA, Goldfine AB, Gordon MB, Creager MA. 2001. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. *Circulation* 103:1816–23
10. Begum N, Ragolia L. 2000. High glucose and insulin inhibit VSMC MKP-1 expression by blocking iNOS via p38 MAPK activation. *Am. J. Physiol. Cell Physiol.* 278:C81–C91
11. Bellows CF, Bernard MJ. 1999. Glutamine is essential for nitric oxide synthesis by murine macrophages. *J. Surg. Res.* 86:213–19
12. Boger RH, Sydow K, Borlak J, Thum T, Lenzen H, et al. 2000. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells. *Circ. Res.* 87:99–105
13. Bogumil R, Knipp M, Fundel SM, Vasak M. 1998. Characterization of dimethylargininase from bovine brain: evidence for a zinc binding site. *Biochemistry* 37: 4791–98
14. Bredt DS. 1999. Endogenous nitric oxide synthesis: biological functions and

pathophysiology. *Free Radical Res.* 31: 577–96
15. Brown AA, Hu FB. 2001. Dietary modulation of endothelial function: implications for cardiovascular disease. *Am. J. Clin. Nutr.* 73:673–86
16. Bulgrin JP, Shabani M, Smith DJ. 1993. Arginine-free diet suppresses nitric oxide production in wounds. *J. Nutr. Biochem.* 4:588–93
17. Cardenas A, Moro MA, Hurtado O, Leza JC, Castrillo A, et al. 2000. Implication of glutamate in the expression of inducible nitric oxide synthase after oxygen and glucose deprivation in rat forebrain slices. *J. Neurochem.* 74:2041–48
18. Castillo L, Beaumier L, Ajami AM, Young VR. 1996. Whole body nitric oxide synthesis in healthy men determined from [$^{15}$N]arginine-to-[$^{15}$N]citrulline labeling. *Proc. Natl. Acad. Sci. USA* 93: 11460–65
19. Castillo L, Sanchez M, Vogt J, Chapman TE, DeRojas-Walker TC, et al. 1995. Plasma arginine, citrulline, and ornithine kinetics in adults, with observations on nitric oxide synthesis. *Am. J. Physiol. Endocrinol. Metab.* 268:E360–E67
20. Chaet MS, Garcia VF, Arya G, Ziegler MM. 1994. Dietary fish oil enhances macrophage production of nitric oxide. *J. Surg. Res.* 57:65–68
21. Chakravarthy U, Hayes RG, Stitt AW, McAuley E, Archter DB. 1998. Constitutive nitric oxide synthase expression in retinal vascular endothelial cells is suppressed by high glucose and advanced glycation end products. *Diabetes* 47:945–52
22. Chan MMY, Mattiacci JA, Hwang HS, Shah A, Fong D. 2000. Synergy between ethanol and grape polyphenols, quercetin, and resveratrol, in the inhibition of the inducible nitric oxide synthase pathway. *Biochem. Pharmacol.* 60: 1539–48
23. Chandler LJ, Guzman NJ, Summers C, Crews FT. 1994. Magnesium and zinc potentiate ethanol inhibition of N-methyl-D-aspartate-stimulated nitric oxide synthase in cortical neurons. *J. Pharmacol. Exp. Ther.* 271:67–75
24. Chang CY, Tucci M, Baker RC. 2000. Lipopolysaccharide-stimulated nitric oxide production and inhibition of cell proliferation is antagonized by ethanol in a clonal macrophage cell line. *Alcohol* 20:37–43
25. Chao CL, Kuo TL, Lee YT. 2000. Effects of methionine-induced hyperhomocysteinemia on endothelium-dependent vasodilation and oxidative status in healthy adults. *Circulation* 101:485–90
26. Chen LG, Wang YP, Kairaitis LK, Wang Y, Zhang BH, Harris DCH. 2001. Molecular mechanisms by which iron induces nitric oxide synthesis in cultured proximal tubule cells. *Exp. Nephrol.* 9:198–204
27. Chen PY, Sanders PW. 1991. L-arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. *J. Clin. Invest.* 88:1559–67
28. Closs EI, Scheld JS, Sharafi M, Forstermann U. 2000. Substrate supply for nitric oxide synthase in macrophages and endothelial cells: role of cationic amino acid transporters. *Mol. Pharmacol.* 57:68–74
29. Colasanti M, Persichini T, Venturini G, Polticelli F, Musci G. 2000. Modulation of the nitric oxide pathway by copper in glial cells. *Biochem. Biophys. Res. Commun.* 275:776–82
30. Courreges MC, Benencia F, Monserrat AJ. 2001. Effect of dietary fish oil on mouse ocular Herpes Simplex type I infection. *Nutr. Res.* 21:229–41
31. Cui L, Takagi Y, Wasa M, Iiboshi Y, Khan J, et al. 1997. Induction of nitric oxide synthase in rat intestine by interleukin-1$\alpha$ may explain diarrhea associated with zinc deficiency. *J. Nutr.* 127:1729–36
32. Darmani H, Harwood JL, Parton J, Jackson SK. 1995. Macrophage activation by lipopolysaccharide, interferon-$\gamma$ and

interleukin-4. Effect of fatty acid metabolism. *Mediator Inflam.* 4:25–30

33. Datta PK, Lianos EA. 1999. Retinoic acids inhibit inducible nitric oxide synthase expression in mesangial cells. *Kidney Int.* 56:486–93
34. Davda RK, Stepniakowski KT, Lu G, Ullian ME, Goodfriend TL, Egan BM. 1995. Oleic acid inhibits endothelial nitric oxide synthase by a protein kinase C-independent mechanism. *Hypertension* 26:764–70
35. Dawes M, Ritter JM. 2000. $Mg^{2+}$-induced vasodilation in human vasculature is inhibited by $N^G$-monomethyl-L-arginine but not by indomethacin. *J. Vasc. Res.* 37:276–81
36. Demeyer GRY, Bult H, Verbeuren TJ, Herman AG. 1992. The role of endothelial cells in the relaxations induced by 13-hydroxylinoleic and 13-hydroperoxylinoleic acid in canine arteries. *Br. J. Pharmacol.* 107:597–603
37. Devaux Y, Grosjean S, Seguin C, David C, Dousset B, et al. 2000. Retinoic acid and host-pathogen interactions: effects on inducible nitric oxide synthase in vivo. *Am. J. Physiol. Endocrinol. Metab.* 279:E1045–E53
38. Diebolt M, Bucher B, Andriantsitohaina R. 2001. Wine polyphenols decrease blood pressure, improve NO vasodilatation, and induce gene expression. *Hypertension* 38:159–65

38a. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. 2001. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *J. Clin. Invest.* 108:1341–48

39. D'Uscio LV, Baker TA, Mantilla CB, Smith L, Weiler D, et al. 2001. Mechanism of endothelial dysfunction in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 21:1017–22
40. Fard A, Tuck CH, Donis JA, Sciacca R, DiTullio MR, et al. 2000. Acute elevation of plasma asymmetric dimethylarginine and impaired endothelial function in response to a high-fat meal in patients with type 2 diabetes. *Arterioscler. Thromb. Vasc. Biol.* 20:2039–44
41. Feron O, Dessy C, Desager JP, Balligand JL. 2001. Hydroxy-methylglutaryl-coenzyme A reductase inhibition promotes endothelial nitric oxide synthase activation through a decrease in caveolin abundance. *Circulation* 103:113–18
42. Fitzpatrick DF, Hirschfield SL, Coffey RG. 1993. Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am. J. Physiol. Heart Circ. Physiol.* 265:H774–H78
43. Freedman JE, Parker C, Li LQ, Perlman JA, Frei B, et al. 2001. Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. *Circulation* 103:2792–98
44. Garcion E, Nataf S, Berod A, Darcy F, Brachet P. 1997. 1,25-Dihydroxyvitamin D-3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis. *Mol. Brain Res.* 45:255–67
45. Ghosh P, Bitsanis D, Ghebremeskel K, Crawford MA, Poston L. 2001. Abnormal aortic fatty acid composition and small artery function in offspring of rats fed a high fat diet in pregnancy. *J. Physiol.* 533:815–22
46. Giovannini L, Migliori M, Longoni BM, Das DK, Bertelli AAE, et al. 2001. Resveratrol, a polyphenol found in wine, reduces ischemia reperfusion injury in rat kidneys. *J. Cardiovasc. Pharmacol.* 37:262–70
47. Goldblatt MI, Choi SH, Swartz-Basile DA, Nakeeb A, Sarna SKS, Pitt HA. 2001. Iron deficiency suppresses ileal nitric oxide synthase activity. *J. Gastrointest. Surg.* 5:393–99
48. Greenberg SS, Xie JM, Wang Y, Kolls J, Shellito J, et al. 1993. Ethanol relaxes

pulmonary artery by release of prostaglandin and nitric oxide. *Alcohol* 10:21–29

49. Grekin RJ, Vollmer AP, Sider RS. 1995. Pressor effects of portal venous oleate infusion. *Hypertension* 26:193–98
50. Grosjean S, Devaux Y, Seguin C, Meistelman C, Zannad F, et al. 2001. Retinoic acid attenuates inducible nitric oxide synthase (NOS2) activation in cultured rat cardiac myocytes and microvascular endothelial cells. *J. Mol. Cell. Cardiol.* 33:933–45
51. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, et al. 1999. Diabetes and cardiovascular disease. *Circulation* 100:1134–46
52. Guo X, Chen LW, Liu WL, Guo ZG. 2000. High glucose inhibits expression of inducible and constitutive nitric oxide synthase in bovine aortic endothelial cells. *Acta Pharmacol. Sin.* 21:325–28
53. Gurujeyalakshmi G, Wang YJ, Giri SN. 2000. Suppression of bleomycin-induced nitric oxide production in mice by taurine and niacin. *Nitric Oxide Biol. Chem.* 4:399–411
54. Han JY, Miura S, Akiba Y, Higuchi H, Kato S, et al. 2001. Chronic ethanol consumption exacerbates microcirculatory damage in rat mesentery after reperfusion. *Am. J. Physiol. Gastrointest. Liver Physiol.* 280:G939–G48
55. Harris HW, Rockey DC, Young DM, Welch WJ. 1999. Diet-induced protection against lipopolysaccharide includes increased hepatic NO production. *J. Surg. Res.* 82:339–45
56. Harris WS, Rambjor GS, Windsor SL, Diederich D. 1997. n-3 fatty acids and urinary excretion of nitrix oxide metabolites in humans. *Am. J. Clin. Nutr.* 65: 459–64
57. Hecker M, Sessa WC, Harris HJ, Anggard EE, Vane JR. 1990. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. USA* 87:8612–16
58. Hernandez-Perera O, Perez-Sala D, Navarro-Antolin J, Sanchez-Pascuala R, Hernandez G, et al. 1998. Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelium-1 and endothelial nitric oxide synthase in vascular endothelial cells. *J. Clin. Invest.* 101:2711–19
59. Hirokawa K, Oshaughnessy KM, Ramrankha P, Wilkins MR. 1994. Inhibition of nitric oxide synthesis in vascular smooth muscle by retinoids. *Br. J. Pharmacol.* 113:1448–54
60. Holmang A, Nilsson C, Niklasson M, Larsson BM, Lonroth P. 1999. Induction of insulin resistance by glucosamine reduces blood flow but not interstitial levels of either glucose or insulin. *Diabetes* 48:106–11
61. Hong CY, Lai LJ, Huang YT. 1995. Endothelium-dependent vasorelaxant effect of trilinolein-mediated by nitric oxide and cyclic-GMP. *Life Sci.* 57:1309–15
62. Houdijk APJ, Visser JJ, Rijinsburger ER, Teerlink T, van Leeuwen PAM. 1998. Dietary glutamine supplementation reduces plasma nitrate levels in rats. *Clin. Nutr.* 17:11–14
63. Hsu JT, Ying CW, Chen CJ. 2000. Regulation of inducible nitric oxide synthase by dietary phytoestrogen in MCF-7 human mammary cancer cells. *Reprod. Nutr. Dev.* 40:11–18
64. Hucks D, Khan NM, Ward JPT. 2000. Essential role of L-arginine uptake and protein tyrosine kinase activity for NO-dependent vasodilation induced by stretch, isometric tension and clyclic AMP in rat pulmonary arteries. *Br. J. Pharmacol.* 131:1475–81
65. Huang A, Vita JA, Venema RC, Keaney JF. 2000. Ascorbic acid enhances endothelial nitric oxide synthase activity by

increasing intracellular tetrahydrobiopterin. *J. Biol. Chem.* 275:17399–406
66. Hung LM, Chen JK, Huang SS, Lee RS, Su MJ. 2000. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc. Res.* 47:549–55
67. Igarashi J, Thatte HS, Prabhakar P, Golan DE, Michel T. 1999. Calcium-independent activation of endothelial nitric oxide synthase by ceramide. *Proc. Natl. Acad. Sci. USA* 96:12583–88
68. Ignarro LJ, Cirino G, Casini A, Napoli C. 1999. Nitric oxide as a signalling molecule in the vascular system: an overview. *J. Cardiovasc. Pharmacol.* 34: 879–86
69. Kamada Y, Nagaretani H, Tamura S, Ohama T, Maruyama T, et al. 2001. Vascular endothelial dysfunction resulting from L-arginine deficiency in a patient with lysinuric protein intolerance. *J. Clin. Invest.* 108:717–24
70. Kanji MI, Toews ML, Carper WR. 1976. A kinetic study of glucose 6-phosphate dehydrogenase. *J. Biol. Chem.* 251: 2258–62
71. Karamsetty MR, Klinger JR, Hill NS. 2001. Phytoestrogens restore nitric oxide-mediated relaxation in isolated pulmonary arteries from chronically hypoxic rats. *J. Pharmacol. Exp. Ther.* 297: 968–74
72. Kasai N, Sugimoto K, Horiba N, Suda T. 2001. Effect of D-glucose on nitric oxide release from glomerular endothelial cells. *Diabetes Metab. Res. Rev.* 17:217–22
73. Kaufman S. 1993. New tetrahydrobiopterin-dependent systems. *Annu. Rev. Nutr.* 13:261–86
74. Keaney JF, Guo Y, Cunningham D, Shwaery GT, Xu A, Vita JA. 1996. Vascular incorporation of $\alpha$-tocopherol prevents endothelial dysfunction due to oxidized LDL by inhibiting protein kinase C stimulation. *J. Clin. Invest.* 98:386–94
75. Kepka-Lenhart D, Mistry SK, Wu G, Morris SM Jr. 2000. Arginase I: a limiting factor for nitric oxide and polyamine synthesis by activated macrophages? *Am. J. Physiol. Integrative Comp. Physiol.* 279:R2237–R42
76. Khair-Eldin T, Sicher SC, Vazquez MA, Chung GW, Stallworth KA, et al. Transcription of the murine iNOS gene is inhibited by docosahexaenoic acid, a major constituent of fetal and neonatal sera as well as fish oil. *J. Exp. Med.* 183:1241–46
77. Khan M, Pahan K, Singh AK, Singh I. 1998. Cytokine-induced accumulation of very long-chain fatty acids in rat C6 glial cells: implications for X-adrenoleukodystrophy. *J. Neurochem.* 71:78–87
78. Kida T, Oku H, Sugiyama T, Ikeda T. 2001. The mechanism and change in the optic nerve head (ONH) circulation in rabbits after glucose loading. *Curr. Eye Res.* 22:95–101
79. Knych ET. 1992. Endothelium-dependent tolerance to ethanol-induced contraction of rat aorta—effects of inhibition of EDRF action and nitric oxide synthesis. *Alcohol. Clin. Exp. Res.* 16:58–63
80. Lawson DL, Mehta JL, Saldeen K, Mehta P, Saldeen TGP. 1991. $\omega$-3 polyunsaturated fatty-acids augmented endothelium-dependent vasorelaxation by enhanced release of EDRF and vasodilator prostaglandins. *Eicosanoids* 4:217–23
81. Lee TJF, Sarwinski S, Ishine T, Lai CC, Chen FY. 1996. Inhibition of cerebral neurogenic vasodilation by L-glutamine and nitric oxide synthase inhibitors and its resersal by L-citrulline. *J. Pharmacol. Exp. Ther.* 276:353–58
82. Li H, Meininger CJ, Hawker JR Jr, Haynes TE, Kepka-Lenhart D, et al. 2001. Regulatory role of arginase I and II in nitric oxide, polyamine, and proline syntheses in endothelial cells. *Am. J. Physiol. Endocrinol Metab.* 280:E75–E82

83. Lissin LW, Cooke JP. 2000. Phytoestrogens and cardiovascular health. *J. Am. Coll. Cardiol.* 35:1403–10
84. Liu Y, Tonna-DeMasi M, Park E, Schuller-Levis G, Quinn MR. 1998. Taurine chloramine inhibits production of nitric oxide and prostaglandin E2 in activated C6 glioma cells by suppressing inducible nitric oxide synthase and cyclooxygenase-2 expression. *Mol. Brain Res.* 59:189–95
85. Locher R, Suter PM, Weisser B, Vetter W. 1997. Low-density lipoprotein-induced formation of nitric oxide by cultured vascular smooth muscle cells of the rat. *Eur. J. Clin. Invest.* 27:603–10
86. Makynen H, Kahonen M, Arvola P, Wuorela H, Vapaatalo H, Porsti I. 1995. Dietary calcium and magnesium supplements in spontaneously hypertensive rats and isolated arterial reactivity. *Br. J. Pharmacol.* 115:1455–62
87. Mannick EE, Bravo LE, Zarama G, Realpe JL, Zhang XJ, et al. 1996. Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in *Helicobacter pylori* gastritis: effect of antibiotics and antioxidants. *Cancer Res.* 56:3238–43
88. Matsuo S, Nakamura Y, Takahashi M, Ouchi Y, Hosoda K, et al. 2001. Effect of red wine and ethanol on production of nitric oxide in healthy subjects. *Am. J. Cardiol.* 87:1029–31
89. Maxwell AJ, Cooke JP. 2001. In *Nitric Oxide and the Cardiovascular System*, ed. J Loscalzo, JA Vita, pp. 547–85. Totowa, NJ: Humana
90. Meininger CJ, Kelly KA, Li H, Haynes TE, Wu G. 2000. Glucosamine inhibits inducible nitric oxide synthesis. *Biochem. Biophys. Res. Commun.* 279:234–39
91. Meininger CJ, Wu G. 1997. L-glutamine inhibits nitric oxide synthesis in bovine venular endothelial cells. *J. Pharmacol. Exp. Ther.* 281:448–53
92. Merrill AL, Schmelz EM. 2001. Sphingolipids: mechanism-based inhibitors of carcinogenesis produced by animals, plants, and other organisms. In *Handbook of Nutraceuticals and Functional Foods*, ed REC Wildman, pp. 377–91. New York: CRC Press
93. Militante JD, Feinstein DL, Syapin PJ. 1997. Suppression by ethanol of inducible nitric oxide synthase expression in C6 glioma cells. *J. Pharmacol. Exp. Ther.* 281:558–65
94. Mohan PF, Desaiah D. 1994. Very-low-density and low-density lipoprotein induce nitric oxide synthesis in macrophages. *Biochem. Biophys. Res. Commun.* 204:1047–54
95. Morales-Ruiz M, Lee MJ, Zollner S, Gratton JP, Scotland R, et al. 2001. Sphingosine1-phosphate activates Akt, nitric oxide production, and chemotaxis through a G(i)-protein/phosphoinositide 3-kinase pathway in endothelial cells. *J. Biol. Chem.* 276:19672–77
96. Morris SM Jr. 1999. Regulation of arginine availability and its impact on NO synthesis. In *Nitric Oxide. Biology and Pathobiology*, ed. LJ Ignarro, pp. 187–97. San Diego, CA: Academic
97. Morris SM Jr. 2002. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu. Rev. Nutr.* 22:87–105
98. Murakami A, Nakashima M, Koshiba T, Maoka T, Nishino H, et al. 2000. Modifying effects of carotenoids on superoxide and nitric oxide generation from stimulated leukocytes. *Cancer Lett.* 149:115–23
99. Mutus B, Rabini RA, Staffolani R, Ricciotti R, Fumelli P, et al. 2001. Homocysteine-induced inhibition of nitric oxide production in platelets: a study on healthy and diabetic subjects. *Diabetologia* 44:979–82
100. Ni ZM, Morcos S, Vaziri ND. 1997. Up-regulation of renal and vascular nitric oxide synthase in iron-deficiency anemia. *Kidney Int.* 52:195–201
101. Nuszkowski A, Grabner R, Marsche G, Unbehaun A, Malle E, Heller R. 2001.

Hypochlorite-modified low density lipoprotein inhibits nitric oxide synthesis in endothelial cells via an intracellular discolation of endothelial nitric oxide synthase. *J. Biol. Chem.* 276:14212–21

102. Ohata T, Fukuda K, Takahashi M, Sugimura T, Wakabayashi R. 1997. Suppression of nitric oxide production in lipopolysaccharide-stimulated macrophage cells by $\omega$-3 polyunsaturated fatty acids. *Jpn. J. Cancer Res.* 88:234–37
103. Okada D. 1996. Zinc protoporphyrin IX suppresses nitric oxide production through a loss of L-arginine in rat cerebellar slices. *Neurosci. Res.* 25:353–58
104. Okuda Y, Kawashima K, Sawada T, Tsurumaru K, Asano M, et al. 1997. Eicosapentaenoic acid enhances nitric oxide production by cultured human endothelial cells. *Biochem. Biophys. Res. Commun.* 232:487–91
105. Omura M, Kobayashi S, Mizukami Y, Mogami K, Todoroki-Ikeda N, et al. 2001. Eicosapentaenoic acid (EPA) induces $Ca^{2+}$-independent activation and translocation of endothelial nitric oxide synthase and endothelium-dependent vasorelaxation. *FEBS Lett.* 487:361–66
106. Pearson PJ, Evora PRB, Seccombe JF, Schaff HV. 1998. Hypomagnesemia inhibits nitric oxide release from coronary endothelium: protective role of magnesium infusion after cardiac operations. *Ann. Thorac. Surg.* 65:967–72
107. Persechini A, McMillan K, Masters BSS. 1995. Inhibition of nitric oxide synthase activity by $Zn^{2+}$ ion. *Biochemistry* 34:15091–95
108. Personett D, Fass U, Panickar K, McKinney M. 2000. Retinoic acid-mediated enhancement of the cholinergic/neuronal nitric oxide synthase phenotype of the medial septal SN56 clone. *J. Neurochem.* 74:2412–24
109. Pollock JS, Forstermann U, Mitchell JA, Warner TD, Schmidt HH, et al. 1991. Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. *Proc. Natl. Acad. Sci. USA* 88:10480–84
110. Pomerantz KB, Hajjar DP, Levi R, Gross SS. 1993. Cholesterol enrichment of arterial smooth muscle cells upregulates cytokine-induced nitric oxide synthesis. *Biochem. Biophys. Res. Commun.* 191:103–9
111. Potter JD, Slattery ML, Bostick RM, Gapstur SM. 1993. Colon cancer: a review of the epidemiology. *Epidemiol. Rev.* 15:499–545
112. Prabhakar SS. 2001. Tetrahydrobiopterin reverses the inhibition of nitric oxide by high glucose in cultured murine mesangial cells. *Am. J. Physiol. Renal Physiol.* 281:F179–F88
113. Ramasamy S, Parthasarathy S, Harrison DG. 1998. Regulation of endothelial nitric oxide synthase gene expression by oxidized linoleic acid. *J. Lipid Res.* 39:268–76
114. Reddy GR, Chetty CS, Sajwan K, Desaiah D. 2001. Lead and manganese alters the activity and expression of nitric oxide synthase in rat brain. *FASEB J.* 15:A222
115. Redmond HP, Wang JH, Bouchier-Hayes D. 1996. Taurine attenuates nitric oxide- and reactive oxygen intermediate-dependent hepatocyte injury. *Arch. Surg.* 131:1280–87
116. Rockett KA, Brookes R, Udalova I, Vidal V, Hill AVS, Kwiatkowski D. 1998. 1,25-dihydroxyvitamin D-3 induces nitric oxide synthase and suppresses growth of *Mycobacterium tuberculosis* in a human macrophage-like cell line. *Infect. Immun.* 66:5314–21
117. Saibara T, Ono M, Iwasaki S, Maeda T, Onishi S, et al. 2001. Effects of ethanol on L-arginine transport in rat Ito cells in relation to nitric oxide production. *Alcohol. Clin. Exp. Res.* 25:39S–45S
118. Sano M, Fujita H, Morita I, Uematsu H, Murota S. 1999. Vitamin K-2 (menatetrenone) induces iNOS in bovine vascular smooth muscle cells: no relationship

between nitric oxide production and $\gamma$-carboxylation. *J. Nutr. Sci. Vitamin.* 45:711–23

119. Schaffer MR, Tantry U, Ahrendt GM, Wasserkrug HL, Barbul A. 1997. Acute protein-calorie malnutrition impairs wound healing: a possible role of decreased wound nitric oxide synthesis. *J. Am. Coll. Surg.* 184:37–43
120. Semon BA, Leung PMB, Rogers QR, Gietzen DW. 1989. Plasma ammonia, plasma, brain and liver amino acids and urea cycle enzyme activities in rats fed ammonium acetate. *J. Nutr.* 119:166–74
121. Shih CL, Chi SI, Chiu TH, Sun GY, Lin TN. 2001. Ethanol effects on nitric oxide production in cerebral pial cultures. *Alcohol. Clin. Exp. Res.* 25:612–18
122. Shikhman AR, Kuhn K, Alaaeddine N, Lotz M. 2001. N-acetylglucosamine prevents IL-1 $\beta$-mediated activation of human chondrocytes. *J. Immunol.* 166:5155–60
123. Shimabukuro M, Ohneda M, Lee Y, Unger RH. 1997. Role of nitric oxide in obesity-induced $\beta$-cell disease. *J. Clin. Invest.* 100:290–95
124. Shimabukuro M, Zhou YT, Levi M, Unger RH. 1997. Fatty acid-induced $\beta$-cell apoptosis: a link betwen obesity and diabetes. *Proc. Natl. Acad. Sci. USA* 95:2498–502
125. Shinozaki K, Nishio Y, Okamura T, Yoshida Y, Maegawa H, et al. 2000. Oral administration of tetrahydrobiopterin prevents endothelial dysfunction and vascular oxidative stress in the aorta of insulin-resistant rats. *Circ. Res.* 87:566–73
126. Shirato M, Sakamoto T, Uchida Y, Nomura A, Ishii Y, et al. 1998. Molecular cloning and characterization of $Ca^{2+}$-dependent inducible nitric oxide synthase from guinea-pig lung. *Biochem. J.* 333:795–99
127. Spranger M, Schwab S, Desiderato S, Bonmann E, Krieger D, Fandrey J. 1998. Manganese augments nitric oxide synthesis in murine astrocytes: a new pathogenetic mechanism in manganism? *Exp. Neurol.* 149:277–83
128. Stephensen CB. 2001. Vitamin A, infection, and immune function. *Annu. Rev. Nutr.* 21:167–92
129. Stroes E, Kastelein J, Cosentino F, Erkelene W, Wever R, et al. 1997. Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. *J. Clin. Invest.* 99:41–46
130. Stroes ESG, van Faassen EE, Yo M, Martasek P, Boer P, et al. 2000. Folic acid reverts dysfunction of endothelial nitric oxide synthase. *Circ. Res.* 86:1129–34

130a. Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP. 2001. Homocysteine impairs the nitric oxide synthase pathway. Role of asymmetric dimethylarginine. *Circulation* 104: 2569–75

131. Sun H, Kaushik PP, Mayhan WG. 2001. Tetrahydrobiopterin, a cofactor for NOS, improves endothelial dysfunction during chronic alcohol consumption. *Am. J. Physiol. Heart Circ. Physiol.* 281:H1863–H69
132. Suschek C, Fehsel K, Kroncke KD, Sommer A, Kolb-Bachofen V. 1994. Primary cultures of rat islet capillary endothelial cell constitutive and cytokine-induced macrophage-like nitric oxide synthases are expressed and activities regulated by glucose concentrations. *Am. J. Pathol.* 145:685–95
133. Swartz-Basile DA, Goldblatt MI, Blaser C, Decker PA, Ahrendt SA, et al. 2000. Iron deficiency diminishes gallbladder neuronal nitric oxide synthase. *J. Surg. Res.* 90:26–31
134. Swierkosz TA, Mitchell JA, Sessa WC, Hecker M, Vane JR. 1990. L-glutamine inhibits the release of endothelium-derived relaxing factor from the rabbit aorta. *Biochem. Biophys. Res. Commun.* 172:143–48
135. Takagawa Y, Berger ME, Hori MT, Tuck

ML, Golub MS. 2001. Long-term fructose feeding impairs vascular relaxation in rat mesenteric arteries. *Am. J. Hypertens.* 14:811–17

136. Tanaka S, Yashiro A, Nakashima Y, Nanri H, Ikeda M. 1997. Plasma nitrite/nitrate level is inversely correlated with plasma low-density lipoprotein cholesterol level. *Clin. Cardiol.* 20:361–65
137. Tian L, Lawrence DA. 1996. Metal-induced modulation of nitric oxide production in vitro by murine macrophages: lead, nickel, and cobalt utilize different mechanisms. *Toxicol. Appl. Pharmacol.* 141:540–47
138. Tolins JP, Shultz PJ, Westberg G, Raij L. 1995. Renal hemodynamic effects of dietary protein in the rat. Role of nitric oxide. *J. Lab. Clin. Med.* 125:228–36
139. Trachtman H, Futterweit S, Crimmins DL. 1997. High glucose inhibits nitric oxide production in cultured rat mesangial cells. *J. Am. Soc. Nephrol.* 8:1276–82
140. Tseng CC, Hattori Y, Kasai K, Nakanishi N, Shimoda SI. 1997. Decreased production of nitric oxide by LPS-treated J774 macrophages in high-glucose medium. *Life Sci.* 60:PL99–106
141. Venkov CD, Myers PR, Tanner MA, Su M, Vaughan DE. 1999. Ethanol increases endothelial nitric oxide production through modulation of nitric oxide synthase expression. *Thromb. Haemost.* 81:638–42
142. Viani P, Giussani P, Riboni L, Bassi R, Tettamanti G. 1999. Sphingosine inhibits nitric oxide synthase from cerebella granule cells differentiated in vitro. *FEBS Lett.* 454:321–24
143. Vishwanathan K, Tackett RL, Stewart JT, Bartlett MG. 2000. Determination of arginine and methylated arginines in human plasma by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B* 748:157–66
144. Wadsworth TL, Koop DR. 1999. Effects of the wine polyphenolics quercetin and resveratrol on proinflammatory cytokine expression in RAW 264.7 macrophages. *Biochem. Pharmacol.* 57:941–49
145. Walker HA, Dean TS, Sanders TAB, Jackson G, Ritter JM, Chowienczyk PJ. 2001. The phytoestrogen genistein produces acute nitric oxide-dependent dilation of human forearm vasculature with similar potency to 17 $\beta$-estradiol. *Circulation* 103:258–62
146. Wan G, Kato N, Watanabe H. 2000. High fat diet elevates the activity of inducible nitric oxide synthase and 1,2-dimethylhydrazine-induced aberrant crypt foci in colon of rats. *Oncol. Rep.* 7:391–95
147. Wan GH, Ohnomi S, Kato N. 2000. Increased hepatic activity of inducible nitric oxide synthase in rats fed on a high-fat diet. *Biosci. Biotech. Biochem.* 64:555–61
148. Wharton BA, Howells GR, McCance RA. 1967. Cardiac failure in kwashiorkor. *Lancet* ii:384–87
149. Williams SB, Goldfine AB, Timimi FK, Ting HH, Roddy MA, et al. 1998. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. *Circulation* 97:1695–701
150. Wu G, Flynn NE, Flynn SP, Jolly CA, Davis PK. 1999. Dietary protein or arginine deficiency impairs constitutive and inducible nitric oxide synthesis by young rats *J. Nutr.* 129:1347–54
151. Wu G, Haynes TE, Li H, Yan W, Meininger CJ. 2001. Glutamine metabolism to glucosamine is necessary for glutamine inhibition of endothelial nitric oxide synthesis. *Biochem. J.* 353:245–52

151a. Wu G, Haynes TE, Yan W, Meininger CJ. 2001. Presence of glutamine:fructose-6-phosphate amidotransferase for glucosamine-6-phosphate synthesis in endothelial cells: effects of hyperglycemia and glutamine. *Diabetologia* 44:196–202

152. Wu G, Meininger CJ. 2000. Arginine

nutrition and cardiovascular function. *J. Nutr.* 130:2626–29

153. Wu G, Meininger CJ, Knabe DA, Bazer FW, Rhoads JM. 2000. Arginine nutrition in development, health and disease. *Curr. Opin. Clin. Nutr. Metab. Care* 3:59–66
154. Wu G, Morris SM Jr. 1998. Arginine metabolism: nitric oxide and beyond. *Biochem. J.* 336:1–17
155. Wu JM, Wang ZR, Hsieh TC, Bruder JL, Zou JG, Huang YZ. 2001. Mechanisms of cardioprotection by resveratrol, a phenolic antioxidant present in red wine. *Int. J. Mol. Med.* 8:3–17
156. Zhai PY, Eurell TE, Cotthaus RP, Jeffery EH, Bahr JM, Gross DR. 2001. Effects of dietary phytoestrogen on global myocardial ischemia-reperfusion injury in isolated female rat hearts. *Am. J. Physiol. Heart Circ. Physiol.* 281:H1223–H32
157. Zhang XH, Li H, Jin HL, Ebin Z, Brodsky S, Goligorsky MS. 2000. Effects of homocysteine on endothelial nitric oxide production. *Am. J. Physiol. Renal Physiol.* 279:F671–F78
158. Yamaguchi K, Honda H, Wakisaka C, Tohei A, Kogo H. 2001. Effects of phytoestrogens on acetylcholine- and isoprenaline-induced vasodilation in rat aorta. *Jpn. J. Pharmacol.* 87:67–73
159. Yoshioka S, Uemura K, Tamaya N, Tamagawa T, Miura H, et al. 2000. Dietary fat-induced increase in blood pressure and insulin resistance in rats. *J. Hypertens.* 18:1857–64