The antihypertensive effect of cysteine

Sudesh Vasdev DVM PhD FICA¹, Pawan Singal PhD DSc², Vicki Gill BBA¹

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Hypertension is a leading cause of morbidity and mortality worldwide. Individuals with hypertension are at an increased risk for stroke, heart disease and kidney failure. Essential hypertension results from a combination of genetic and lifestyle factors. One such lifestyle factor is diet, and its role in the control of blood pressure has come under much scrutiny. Just as increased salt and sugar are known to elevate blood pressure, other dietary factors may have antihypertensive effects. Studies including the Optimal Macronutrient Intake to Prevent Heart Disease (OmniHeart) study, Multiple Risk Factor Intervention Trial (MRFIT), International Study of Salt and Blood Pressure (INTERSALT) and Dietary Approaches to Stop Hypertension (DASH) study have demonstrated an inverse relationship between dietary protein and blood pressure. One component of dietary protein that may partially account for its antihypertensive

effect is the nonessential amino acid cysteine. Studies in hypertensive humans and animal models of hypertension have shown that N-acetylcysteine, a stable cysteine analogue, lowers blood pressure, which substantiates this idea. Cysteine may exert its antihypertensive effects directly or through its storage form, glutathione, by decreasing oxidative stress, improving insulin resistance and glucose metabolism, lowering advanced glycation end products, and modulating levels of nitric oxide and other vasoactive molecules. Therefore, adopting a balanced diet containing cysteine-rich proteins may be a beneficial lifestyle choice for individuals with hypertension. An example of such a diet is the DASH diet, which is low in salt and saturated fat; includes whole grains, poultry, fish and nuts; and is rich in vegetables, fruits and low-fat dairy products.

Key Words: *Advanced glycation end products; Cysteine; Hypertension; Insulin resistance; Nitric oxide; Oxidative stress*

Essential hypertension affects over 600 million people worldwide (1). It is a vascular disease characterized by increased blood pressure, insulin resistance, oxidative stress and endothelial dysfunction (2-11). A combination of genetic and lifestyle factors is thought to cause hypertension (Figure 1). Diet is one lifestyle factor that has come under much scrutiny. Just as certain dietary factors, such as increased salt and sugar or deficiencies in antioxidants, are known to increase blood pressure (1,12-17), other components of diet may have antihypertensive effects. The Dietary Approaches to Stop Hypertension (DASH) study compared a typical North American diet with the DASH diet, which is rich in vegetables, fruits and low-fat dairy products; low in salt and saturated fat; and included whole grains, poultry, fish, and nuts (18). The DASH diet lowered blood pressure compared with the typical North American diet. When these two diets were modified to contain a similar reduced sodium content, the DASH diet again lowered blood pressure more than the control diet (13). This indicated that a dietary factor other than reduced salt content was having an antihypertensive effect. The DASH diet contained a higher protein content than the North American diet (17.9% versus 13.8%). Several other studies, including the Optimal Macronutrient Intake to Prevent Heart Disease (OmniHeart) study (19), Multiple Risk Factor Intervention Trial (MRFIT) (20) and International Study of Salt and Blood Pressure (INTERSALT) (21), have also demonstrated an inverse relationship between dietary protein and blood pressure. We suggest that the nonessential amino acid cysteine may contribute to this antihypertensive effect.

Several research reports support an antihypertensive role for cysteine. Studies using dietary supplementation of the cysteine analogue N-acetylcysteine (NAC) have shown that, overall, it

prevents or attenuates increased blood pressure in animal models of hypertension (22-33). Although the cysteine precursor methionine increases the cardiovascular risk factor homocysteine and increases blood pressure in normal rats (34-36), it has been shown to lower blood pressure in hypertensive rats (35,37). Additionally, in human studies, using NAC as an adjunct to other antihypertensive therapies results in a decrease in blood pressure (38-40).

The present review focuses on the normal physiological role of cysteine, examines the evidence of its antihypertensive effects in animal and human studies, and discusses the potential mechanisms by which it exerts its antihypertensive effect.

PHYSIOLOGICAL FUNCTIONS OF CYSTEINE

Cysteine, a nonessential amino acid, is found in foods such as meats, fish, whole grains, soybeans and legumes (41) (Table 1), and can be formed endogenously via metabolism of its precursor, the essential amino acid methionine (Figure 2). Although nonessential from a dietary perspective, cysteine participates in several essential biological processes.

The chemical structure of cysteine contains a free sulfhydryl (SH) group (Figure 3), and it is this reactive entity that contributes to much of cysteine's biological activity. Cysteine is a component of many structural and functional proteins. One of the most important characteristics of cysteine is its ability to stabilize protein structures by forming disulfide linkages with other cysteine molecules. These covalent cross-links add stability to the three-dimensional structures of protein. This conformational control influences the properties of the protein – for instance, its susceptibility to denaturation (42). This characteristic contributes to maintaining the integrity of vascular structures. The precise location of cysteine within a protein

¹Discipline of Medicine, Faculty of Medicine, Health Sciences Centre, Memorial University, St John's, Newfoundland; ²Institute of Cardiovascular Sciences, St Boniface General Hospital Research Centre, University of Manitoba, Winnipeg, Manitoba

Correspondence and reprints: Dr Sudesh Vasdev, Room H-4310, Health Sciences Centre, Memorial University, St John's, Newfoundland A1B 3V6. Telephone 709-777-7260, fax 709-777-7010, e-mail svasdev@mun.ca

Figure 1 Mechanism of hypertension. Hypertension develops from a combination of genetic and lifestyle factors such as diet. A diet high in salt or sugar, and low in antioxidants and protein, has been implicated in hypertension. Increased oxidative stress and a decreased bioavailability of nitric oxide (NO); insulin resistance and altered glucose metabolism with an increase in advanced glycation end products (AGEs); activation of the renin-angiotensin system (RAS); and damage to kidneys resulting in altered renal function are all mechanisms that contribute to the development of hypertension

also plays a direct role in the protein's function. For example, cysteine is found at the active site of several enzymes, including the vascular enzyme endothelial nitric oxide (NO) synthase (eNOS) (43), and regulates catalytic activity. As a moderately powerful redox pair, cysteine and its disulfide partner cystine have an important physiological function as antioxidants. Additionally, cysteine is a rate-limiting substrate in the biosynthesis of reduced glutathione, a tripeptide, which also contains glutamate and glycine (Figure 3). Here again, the free SH group of cysteine within glutathione confers functional properties to this molecule. Reduced glutathione/oxidized glutathione (GSH/GSSG) is a ubiquitous and powerful antioxidant pair found in all cells of the body (Table 2). GSH is involved in the enzymatic reduction (catalyzed by glutathione peroxidase) of hydrogen peroxide (H_2O_2) and lipid hydroperoxides. Due to its high redox potential, GSH plays a major role in regenerating other antioxidants to their reduced states, consequently increasing overall antioxidant capacity (44-46). GSH is an essential component of the glyoxalase enzyme system, which is responsible for catabolism of the highly reactive aldehydes methylglyoxal and glyoxal (47). Glutathione and cysteine also

TABLE 1
Dietary sources of cysteine

Food	Serving	Cysteine (mg)
Meat		
Lamb, leg and shoulder, braised	3.5 oz (100 g)	402
Chicken breast, without skin, roasted	½ breast (86 g)	341
Pork, Boston blade, roasted	3.5 oz (100 g)	316
Beef, chuck roast, braised	3.5 oz (100 g)	370
Fish		
Salmon, baked	3 oz (85 g)	232
Halibut, baked	3 oz (85 g)	243
Tuna, canned, light	3 oz (85 g)	232
Grains		
Barley, pearled, light	½ cup (100 g)	219
Oat bran, cooked	½ cup (110 g)	108
Whole wheat flour	1 cup (120 g)	381
Quinoa	½ cup (85 g)	456
Soybean products		
Soybean flour, defatted	1 cup (100 g)	757
Soy milk	1 cup (240 g)	113
Soybeans, boiled	1 cup (172 g)	461
Tofu, raw, firm	½ cup (126 g)	275
Dairy		
Cottage cheese, low-fat	1 cup (226 g)	287
Cow milk, 1%	8 oz (244 g)	74
Chicken eggs, boiled	1 large (50 g)	146
Other		
Almonds, blanched	1 oz (28 g)	133
Split peas, boiled	1 cup (196 g)	249
Broadbeans, boiled	1 cup (170 g)	165
Figs, dried	10 (187 g)	94
Avocado, medium	1 (304 g)	52

Data from reference 41

bind these aldehydes, causing them to be excreted in bile and urine (48).

Glutathione comprises 90% of the total nonprotein low molecular weight thiols in the body. Together with cysteine, glutathione forms part of an endogenous thiol pool that reacts with the vasoregulatory molecule NO to form nitrosothiol, thus stabilizing this normally volatile molecule (49-51). In this way, nitrosothiol increases the bioavailability of NO and potentiates NO-mediated effects, such as vasorelaxation, as well as antiaggregatory and anti-inflammatory processes. Nitrosothiol also has independent biological activity, similar to that of NO (49,52-55). In a later section of the present review, S-nitrosothiols (SNOs) are discussed in detail.

Proteins and amino acids are also sources of energy. Amino acids stimulate insulin secretion, which subsequently leads to their cellular uptake and protein synthesis (56). It was demonstrated earlier that cysteine also has insulin-like actions, such as promoting glucose uptake into adipose cells, an action mediated by its free SH groups (57). Cysteine has been subsequently shown to increase the levels of glucose transporter (GLUT) 3 and GLUT 4 (58). Thus, cysteine likely plays a role in balancing energy metabolism.

To summarize, cysteine influences structure and function of proteins, and acts both directly and indirectly through glutathione to control oxidative stress, maintain reactive aldehydes at low levels, increase bioavailability of NO, and influence insulin and glucose metabolism. Because various proteins and

Figure 2) Metabolic formation of cysteine. Methionine, an essential amino acid obtained through diet, is converted via an enzymatic pathway to the thiol-containing amino acid cysteine. This pathway includes the vitamin B₆-dependent enzymes cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE). N-acetylcysteine (NAC), a cysteine analogue also available in the diet, is deacylated to cysteine primarily in the kidneys. Cysteine may be further converted to the ubiquitous antioxidant glutathione (GSH) or enzymatically converted into the vasoactive molecule H₂S via CBS

processes involved in vascular function are influenced by oxidative, carbonyl or nitrosative stimuli (43,59-67), cysteine potentially plays an important role in regulating blood pressure. This indicates that a disruption in cysteine or glutathione availability, or an increased demand on these thiols, contributes to the etiology of hypertension. It further suggests that supplementary agents such as NAC, which increase endogenous levels of these thiols, or intake of dietary NAC or cysteine-containing proteins, may be useful in controlling high blood pressure. The following section presents the results of various animal (Table 3) and human (Table 4) studies that investigated the effects of cysteine supplementation on blood pressure. Throughout the present review, doses of NAC or methionine are given at the first occurrence of the citation.

EFFECT OF CYSTEINE ON BLOOD PRESSURE

Animal studies

Because cysteine is rapidly oxidized in the air, it can not be added, as is, to animal diet or drinking water. NAC, a cysteine analogue found naturally in several fruits and vegetables (68), is stable and also available as a commercial preparation. In the body, NAC is deacylated, primarily in the kidneys, to produce cysteine. Many studies investigating the effect of cysteine use either NAC or methionine, the metabolic precursor to cysteine. Rats are used to model human hypertension, and although these models are not perfect, they do generally represent disease conditions, and are most convenient for studying in vivo mechanisms of disease and response to treatments.

The following studies investigated the antihypertensive effect of NAC in spontaneously hypertensive rats (SHRs), a genetic model of hypertension. In an acute study of the angiotensin-converting enzyme (ACE) inhibitors captopril and enalaprilat,

Figure 3) Chemical structures of cysteine and reduced glutathione. Free sulfhydryl groups of these molecules contribute to much of their biological activity

TABLE 2

Redox potentials of antioxidants in mammalian oxidation systems

Redox pair	E'° (V)
O ₂ /H ₂ O	+0.82
Vitamin E oxidized/reduced	+0.37
Ubiquinone oxidized/reduced	+0.10
Vitamin C oxidized/reduced	+0.08
Cystine/cysteine	-0.22
GSSG/GSH	-0.24
Lipoate oxidized/reduced	-0.29
NAD ⁺ /NADH	-0.32
H ⁺ /H ₂	-0.42

Redox pairs given by increasing electronegativity and antioxidant activity. E'° Standard reduction potential; GSSG/GSH Glutathione oxidized/reduced

intravenous pretreatment with 300 mg/kg body weight (bw) of NAC was shown to potentiate the blood pressure-lowering effects of these traditional antihypertensive drugs in SHRs (22). Oral NAC treatment of 4 g/kg bw/day in drinking water for two weeks was shown to reduce systolic but not diastolic blood pressure (24), while the same dose given for four weeks reduced mean arterial blood pressure in this model (25). A study using a lower dose of NAC, 1.5 g/kg bw/day in drinking water for six weeks, showed no effect on blood pressure (69). However, an even lower dose of NAC, 760 mg/kg bw/day in the diet for a longer interval of 11 weeks, normalized blood pressures in these animals (23). These studies analyzed the ability of NAC to treat SHRs that were already hypertensive. One study (26) in young prehypertensive SHRs demonstrated that chronic NAC treatment with 1.5 g/kg bw/day in drinking water for eight weeks attenuated the rise in blood pressure. On the whole, these studies showed that NAC treatment has antihypertensive effects in this genetic model of hypertension.

Dahl salt-sensitive (DSS) rats are salt-sensitive models of hypertension – their blood pressure rises with increasing levels of dietary salt. Studies of NAC supplementation in this model are scant. NAC, 15 g/L in drinking water for four weeks, prevented an increase in systolic blood pressure in rats

TABLE 3
The effect of cysteine on blood pressure (BP) in animal models of hypertension

Animal model	Dose/duration	Effect on BP (reference)
SHRs given ACE inhibitors (captopril or enalapril)	300 mg intravenous NAC/kg bw	Potentiated the antihypertensive effect of concomitant ACE inhibitor (22)
SHRs	760 mg NAC/kg bw/day for 11 weeks in drinking water	Normalized elevated SBP (23)
	4 g NAC/kg bw/day for 2 weeks in drinking water	Decreased SBP but not DBP (24)
	4 g NAC/kg bw/day for 4 weeks in drinking water	Decreased MAP (25)
	1.5 g NAC/kg bw/day for 6 weeks in drinking water	No effect on SBP (67)
	1.5 g NAC/kg bw/day for 8 weeks in drinking water	Prevented the rise in SBP, DBP and MAP in young SHRs; no effect in adult SHRs (26)
	500 mg methionine/kg bw/day for 10 weeks in diet	Increased SBP in control WKY rats and attenuated an increase in SHRs (35)
DOCA-treated SD rats	500 mg methionine/kg bw/day for 10 weeks in diet	Increased BP in control SD rats and attenuated a rise in SBP in DOCA rats (37)
DSS rats treated with high-salt diet	15 g NAC/L drinking water for 4 weeks	Prevented an increase in SBP (27)
	4 g NAC/kg bw/day for 5 weeks in diet	Prevented an increase in MAP (28)
	10 g NAC/L drinking water for 4 weeks	Prevented an increase in MAP (29)
WKY rats treated with 4% fructose in drinking water	664 mg NAC/kg bw/day for 11 weeks in diet	Prevented an increase in SBP (30)
SD rats treated with 60% fructose in drinking water	1.5 g NAC/kg bw/day for 12 weeks in drinking water	Prevented an increase in SBP and DBP (31)
L-NAME-treated Wistar rats	1.5 g NAC/kg bw/day for 4 weeks in drinking water	Prevented the L-NAME-induced increase in MAP (32)
	1.5 g NAC/kg bw/day for 5 weeks in drinking water	Attenuated the L-NAME-induced increase in MAP (33)

ACE Angiotensin-converting enzyme; bw Body weight; D Diastolic; DOCA Deoxycorticosterone acetate salt; DSS Dahl salt-sensitive; L-NAME N-(omega)-nitro-L-arginine methyl ester; MAP Mean arterial pressure; NAC N-acetylcysteine; S Systolic; SD Sprague-Dawley, SHR Spontaneously hypertensive rat; WKY Wistar-Kyoto

TABLE 4
Effect of cysteine on blood pressure (BP) in humans with hypertension

Human subjects	Dose/duration	Effect on BP (reference)
Hypertensive subjects receiving ACE inhibitor lisinopril	1.2 g/day oral NAC for 1 week	Decreased SBP and DBP (38)
Hypertensive subjects who smoked and were receiving ACE inhibitors captopril or enalapril	1.8 g/day oral NAC for 3 weeks	Decreased 24 h ambulatory and daytime SBP and DBP (39)
Hypertensive subjects with type 2 diabetes whose previous antihypertensive medications were discontinued 15 days before study	1.2 g/day NAC plus 1.2 g/day arginine orally for 6 months	Decreased SBP, DBP and mean arterial BP (40)

ACE Angiotensin-converting enzyme; D Diastolic; NAC N-acetylcysteine; S Systolic

fed a high-salt diet (8% NaCl) (27). In a five-week study of rats on a high-salt diet, 4 g/kg bw/day of NAC in the diet prevented an increase in mean arterial blood pressure (28). In an abstract, Kunes et al (29) reported that 10 g/L of NAC in drinking water for four weeks prevented hypertension in young rats. Although few in number, these studies concur in their findings that NAC effectively prevented a salt-induced increase in blood pressure in this model.

Fructose-fed rats represent a model of diet-induced or acquired hypertension. Wistar-Kyoto (WKY) rats fed 4% fructose in drinking water for 11 weeks developed an increase in systolic blood pressure, which was prevented by 664 mg/kg bw/day of NAC in the diet (30). NAC, 1.5 g/kg bw/day in drinking water for 12 weeks, also prevented an increase in systolic and diastolic blood pressures in Sprague-Dawley (SD) rats given 60% fructose (31). These two studies agree in their results, adding to the

weight of evidence indicating that cysteine lowers blood pressure.

Hypertension is associated with a reduced bioavailability of the vasodilator NO with consequent endothelial dysfunction (9,11). A model of hypertension that seeks to mimic these conditions is the N-(omega)-nitro-L-arginine methyl ester (L-NAME)-treated rat. L-NAME inhibits NO synthase (NOS), the enzyme that catalyzes the formation of NO, causing a reduction in NO-mediated vasodilation. L-NAME treatment in Wistar rats for four weeks caused an increase in mean arterial pressure that was prevented by NAC supplementation of 1.5 g/kg bw/day in drinking water (32). In a similar five-week study, NAC attenuated the development of L-NAME-induced hypertension (33). The concurrence of these studies suggests that NAC compensates for the L-NAME-induced loss of NO to reduce blood pressure in this model.

The essential amino acid methionine is metabolized endogenously to cysteine (Figure 2). However, homocysteine, a cardiovascular disease risk factor (34), is also increased when dietary methionine is increased (35,36). In one study, intake of a diet supplemented with 500 mg/kg bw/day of methionine for 10 weeks caused similar elevations in plasma homocysteine in normotensive WKY control rats and SHRs. However, it resulted in a moderate increase in systolic blood pressure in WKY control rats, while it attenuated the increase in blood pressures in SHRs (35). Similarly, these authors reported that methionine supplementation increased blood pressure in another type of normotensive control rat, SD rats, while attenuating a blood pressure increase in a deoxycorticosterone acetate (DOCA) salt hypertensive rat model (37). These reports of opposite effects on blood pressure in normotensive and hypertensive animals, combined with the potential increased risk of cardiovascular disease, may preclude the use of methionine supplementation for the control of blood pressure

in humans. However, this research contributes to the body of data demonstrating the blood pressure-lowering effect of cysteine in hypertensive models. Collectively, these studies in various rat models of hypertension provide evidence that cysteine lowers blood pressure or prevents its increase.

Human studies

There are no reported studies using NAC as a monotherapy in humans with hypertension. However, in one study of six hypertensive subjects exhibiting fairly good blood pressure control (average of 139/93 mmHg) with the ACE inhibitor lisinopril, the addition of NAC, 1.2 g/day for one week, caused a significant decrease in both the systolic and diastolic blood pressures (38). In a second study, 18 hypertensive smokers whose blood pressures were not controlled with ACE inhibitor monotherapy (enalapril or captopril) were given adjunct treatment with NAC, 1.8 g/day for 21 days, resulting in a decrease in 24 h ambulatory and daytime systolic and diastolic blood pressures (39). Because neither of these studies included subjects treated with only NAC, we cannot discern whether NAC potentiated the effect of the ACE inhibitors, or whether there was an independent additive antihypertensive effect of NAC. A third study examined the effect of a combination of NAC, 1.2 g/day, and an equal dose of arginine, the substrate of NOS, in a group of 12 type 2 diabetic patients with hypertension (40). Traditional antihypertensive therapy was stopped and followed by a 15-day washout period before the combination treatment was started. Compared with basal levels, systolic, diastolic and mean arterial pressures were reduced after six months of combined treatment. This combination treatment using the NOS substrate and an antioxidant may work by concomitantly increasing NO production and preserving its destruction by reactive oxygen species (ROS). Of note, none of these studies in humans reported any adverse effects of NAC. Studies using NAC as a monotherapy are necessary to confirm its antihypertensive effect in humans.

POTENTIAL MECHANISMS OF ANTIHYPERTENSIVE ACTION

The preponderance of evidence from these animal and human studies demonstrates the antihypertensive effect of cysteine. The potential mechanisms of antihypertensive action of cysteine are discussed next (Figure 4).

Cysteine lowers oxidative stress

An imbalance in which ROS or reactive nitrogen species (RNS) outweigh antioxidant capacity is known as oxidative stress. This may occur due to an increased production of reactive species or a diminished neutralizing capacity, or both. Reactive species are generated from various sources within the body. NO itself is a reactive molecule that gives rise to RNS including peroxynitrite and peroxynitrous acid, which are unstable and result in the production of hydroxyl radicals ($\bullet\text{OH}$) (70). This not only increases oxidative stress but also decreases the bioavailability of NO. In vascular tissue, the membrane enzyme NADPH oxidase is a major source of superoxide radical (O_2^-), which further gives rise to radicals such as H_2O_2 and $\bullet\text{OH}$ (71). These reactive species are controlled enzymatically by superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase. They are also controlled

Figure 4 Sources and antihypertensive actions of cysteine and glutathione (GSH). Cysteine is derived from dietary methionine, cysteine or N-acetylcysteine. It exerts its antihypertensive action directly, or indirectly through its storage form GSH. Cysteine decreases blood pressure by lowering oxidative stress, improving insulin resistance, altering glucose metabolism to lower advanced glycation end products (AGEs), improving the bioavailability of nitric oxide (NO), modulating other vasoactive molecules such as angiotensin II and H_2S , and improving renal function

nonenzymatically by vitamins such as vitamin E and C, or by thiol compounds including cysteine and glutathione.

Under normal circumstances, ROS and RNS may play a role in vascular homeostasis. They are involved in control of intracellular calcium levels (59-61); activity of vascular enzymes including NOS (43) and ACE (62,72), and glycolytic enzymes such as glyceraldehyde 3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase (63,73,74); function of membrane receptors and cell signalling activity (75-79); and gene transcription (64). Thus, when there is oxidative stress, dysfunction of these proteins may result in altered cell signalling and protein expression (64,79), decreased NO (7,80) and increased angiotensin II (AII) (62), altered glucose metabolism (73) and increased intracellular calcium (60,81). This can result in changes characteristic of hypertension, including insulin resistance, increased cytosolic free calcium, vasoconstriction and peripheral vascular resistance, and vascular smooth muscle cell (VSMC) proliferation.

Oxidative stress is increased in animal models and humans with hypertension. SHR, DSS rats, and fructose- and salt-induced hypertensive rats all show an increase in oxidative stress and a reduction in antioxidant capacity (27,31,82-86). Humans with essential hypertension show an elevation in O_2^- and H_2O_2 production by polymorphonuclear cells (4,7,8), an increase in lipid peroxidation parameters in serum or plasma (5,6,8,87-89), and a decline in antioxidant capacity (5,6,87,89).

Cysteine may regulate blood pressure in part by acting as an antioxidant, either directly or via glutathione to reduce oxidative stress. As previously discussed, cysteine/cystine and GSH/GSSG are powerful redox pairs that are directly involved in

neutralizing reactive species and increasing antioxidant capacity. NAC has also been shown to reduce oxidative stress in isolated cells, and in animals and humans. During *in vitro* testing, NAC was a powerful scavenger of the reactive species HOCl and $\bullet\text{OH}$; it also reacted slowly with H_2O_2 and not at all with O_2^- (90). However, another study showed that NAC added *in vitro* to neutrophils of healthy subjects inhibited chemiluminescence, a measure of superoxide production (91). NAC decreased lipid peroxidation in liposomes of bovine brain extract by 50% (92). A cell culture study using T lymphocytes from children with chronic renal failure showed that incubation with NAC decreased intracellular oxidative stress and ROS-mediated apoptosis (93). Addition of NAC to isolated rabbit heart increased glutathione content in a dose-dependent manner and protected against oxidative ischemic reperfusion injury (94).

In *in vivo* animal studies of hypertension, NAC not only reduced blood pressure but also was effective at reducing oxidative stress and improving antioxidant capacity. NAC treatment increased aortic GSH content, lowered the GSSG/GSH ratio and malondialdehyde levels, and protected against peroxynitrite-induced endothelial dysfunction in both WKY rats and SHR (24). In SHR, treatment with NAC decreased conjugated dienes, a measure of oxidative stress, in the left ventricle and kidney (26). In young SHR, a combination of NAC, 200 mg/kg bw/day, given intraperitoneally, and tempol, the SOD mimetic, 250 mg/kg bw/day given intraperitoneally for one week, lowered renal nitrotyrosine levels, an indicator of tissue oxidative and nitrosative stress (82). Although a diet supplemented with methionine did not increase plasma cysteine levels in SHR, it did increase plasma GSH levels (35). In high salt-treated DSS rats, supplementation with NAC diminished renal NADPH oxidase activity and expression, and lowered urinary levels of H_2O_2 and 8-isoprostanes, a marker of lipid peroxidation (27). Another study in this model showed that NAC treatment lowered superoxide release and increased the GSH/GSSG ratio in the kidneys (28). NAC supplementation with 1.44 g/kg bw/day in drinking water for eight weeks in type 1 diabetic rats reduced plasma and myocardial free 15- F_{2t} -isoprostanes, and increased plasma SOD activity and GSH (95). A similar result was found in fructose-fed hypertensive rats, in which NAC supplementation abolished the increase in plasma 15- F_{2t} -isoprostanes (31). In high sucrose-fed Wistar rats, addition of oral NAC, 2 mg/L in drinking water, increased SOD and GSH levels, glutathione peroxidase activity and the GSH/GSSG ratio (85). NAC given to L-NAME-treated rats prevented an increase in superoxide production in the aorta and conjugated diene levels in the left ventricle and kidney (32). Another study in this rat model showed that two weeks of supplementation with 75 mg/day of NAC in drinking water normalized cardiac glutathione levels (96).

Although studies assessing the effect of NAC on oxidative stress and antioxidant capacity in humans are few, those that have been documented show positive results. In a study of healthy subjects, a single 400 mg oral dose of NAC lowered neutrophil chemiluminescence (97). Oral NAC, 1.2 g/day for four weeks, given to type 2 diabetic patients increased intrathyroidal GSH levels and the GSH/GSSG ratio (98). In type 2 diabetic hypertensive patients, a combination of NAC and L-arginine supplementation lowered plasma nitrotyrosine

(40). Taken together, these experimental and human studies demonstrate that cysteine decreases oxidative stress and increases antioxidant capacity.

Cysteine improves insulin resistance

Insulin is a hormone that regulates carbohydrate and lipid metabolism, and stimulates protein synthesis. The effects of insulin are initially mediated by the binding of the hormone to the insulin receptors on the cell surface. The tyrosine kinase activity of the receptor then stimulates a number of intracellular signalling cascades known as insulin signalling. This signalling not only promotes glucose uptake but modulates other functions involved in blood pressure regulation, such as NO-mediated vasodilation (99-102), AII signalling (103,104) and antinatriuretic activity (105).

Insulin resistance is characterized by inadequate glucose uptake with altered glucose metabolism in peripheral tissues at a given concentration of plasma insulin (2). There is increasing evidence that insulin resistance plays a key role in essential hypertension in humans (2,3,106). Abnormalities in glucose use are estimated to exist in 25% of the general population and in up to 80% of subjects with essential hypertension (2,107-109). In humans with essential hypertension, insulin resistance involves impairment of the nonoxidative (glycolytic) pathways of intracellular glucose metabolism (2). Insulin resistance appears to be a primary defect because it exists in normotensive individuals at risk for essential hypertension (110). Studies show the presence of insulin resistance in SHR at a young age (111,112) and before development of hypertension (113). Insulin resistance is also a characteristic of salt-sensitive rats and humans with hypertension (114-118). In genetic, salt- and sugar-induced rat models of hypertension, there is impairment in the insulin receptor substrate (IRS)/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) and IRS/PI3K/Akt/eNOS insulin signalling pathways (86,117-120). ROS have been shown to inhibit insulin signalling in VSMCs (79).

Cysteine may exert antihypertensive effects by improving insulin resistance and glucose metabolism. An early study demonstrated that cysteine has an insulin-like action, promoting the entry of glucose into adipose cells, mediated by its free SH group (57). GLUT 3 and GLUT 4 were increased, and a marked enhancement of glucose uptake was demonstrated, in mouse soleus muscle and human neuroblastoma cells when exposed to cysteine (58). NAC significantly reduced high sucrose-induced hyperglycemia in Wistar rats (85). Increasing the cysteine-containing protein content in the diet or supplementing the diet with NAC, 5.8 g/kg or 20 g/kg of food, prevented oxidative stress and insulin resistance in sucrose-fed Wistar rats (86). Fructose-induced hypertensive SD rats showed an increase in plasma insulin and a decrease in insulin sensitivity that was attenuated by NAC in diet (31). Similarly, another study in fructose-treated WKY rats showed that NAC normalized elevated plasma glucose and insulin (30). Intravenous administration of NAC, 0.5 mg/kg to 2 mg/kg bw, increased glucose use during a hyperglycemic clamp test in healthy volunteers (121). The homeostasis model assessment, an index of insulin sensitivity, was reduced in a group of obese, nondiabetic patients. Supplementation with 600 mg/day NAC for eight weeks increased the homeostasis model assessment in these patients (122). Treatment with 1.8 g/day to 3 g/day NAC for

five to six weeks also improved insulin sensitivity in women with polycystic ovarian syndrome, another condition with insulin resistance (123). It has also been suggested that cysteine may improve glucose metabolism by preventing oxidative or nitrosative inhibition of the glycolytic enzymes glucose-6-phosphate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase (74,124).

Cysteine lowers advanced glycation end products

Insulin resistance and impaired glucose metabolism can result in an accumulation of reactive aldehydes, including methylglyoxal and glyoxal. These aldehydes react with free SH and amino groups of proteins and DNA to form advanced glycation end products (AGEs) (48,125-128). These structural changes alter protein function (65,129-134). AGEs may also interact with membrane receptors, including receptors of AGEs (RAGEs) and scavenger receptors, to alter cell function (135,136). Oxidative stress has also been shown to increase aldehyde and AGE formation (137,138).

Methylglyoxal and glyoxal are normally maintained at a low level because they are catabolized via the glutathione-dependent glyoxalase enzyme system. Thiols such as cysteine also bind these aldehydes, allowing them to be excreted in bile and urine (48,139). Like ROS, at low levels, AGEs may have normal physiological roles (140). However, when AGE levels increase, there may be pathological consequences. A growing body of evidence implicates AGEs in the development of hypertension (16,23,30,141-147). Cells exposed to aldehydes or AGEs show an increase in intracellular ROS (147-150), changes in calcium handling (66,135), altered cell signalling with increased transcription of inflammatory proteins and production of vasoconstricting agents (150-154), and VSMC proliferation (155,156). Rats treated with methylglyoxal develop an increase in tissue AGEs, platelet cytosolic free calcium and blood pressure, as well as adverse renal vascular changes (157). Additionally, methylglyoxal and AGEs are increased in both genetic and diet-induced rat models of hypertension (16,23,30,141-144,146). Although there is increasing evidence of a role for AGEs in animal models of hypertension, there are few studies in humans. One study of hypertensive patients showed an elevation in plasma AGEs in association with increased vascular stiffness (158). Another study in diabetic patients showed that those with hypertension had higher plasma AGE levels than their normotensive counterparts (159). Aldehydes and AGEs have also been shown to inhibit glycolytic enzymes (65,134,160), alter insulin function (132) and impair insulin signalling (130,131), as well as inhibit antioxidant enzymes (65,129), thus perpetuating the cycle of insulin resistance and oxidative stress.

Cysteine may attenuate hypertension by boosting GSH-dependent catabolism and excretion of reactive aldehydes, thereby preventing AGE formation and the subsequent downstream effects. Most of the research on cysteine and AGEs has focused on their ability to prevent insulin resistance, although some studies have investigated their effect on oxidative stress, intracellular calcium levels or cell signalling. Unoki et al (161) demonstrated that AGE-RAGE interaction increased intracellular oxidative stress, causing a decrease in glucose uptake in cultured 3T3-L1 adipocytes. This effect was completely reversed by treatment with NAC. AGE-RAGE binding caused an

increase in oxidative stress and AGE production in rat mesangial cells that was prevented by addition of NAC (153). NAC attenuated a methylglyoxal-induced increase in the transcription factor nuclear factor kappa B and prevented an increase in GSSG in the VSMC of SHR (147). Jia and Wu (131) showed that methylglyoxal decreased insulin-induced IRS-1 tyrosine phosphorylation and decreased kinase activity of PI3K to impair insulin signalling in cultured 3T3-L1 adipocytes. This impairment was prevented by the addition of NAC to the cell culture. These authors also demonstrated that 10 mg/kg bw/day of NAC given to fructose-fed SD rats in drinking water for nine weeks prevented an increase in serum and adipose tissue methylglyoxal levels, and improved glucose uptake (131). Coinfusion of NAC with high amounts of glucose in SD rats prevented sugar-induced insulin resistance and lowered carbonyl content in soleus muscles (162). Supplementation with NAC decreased tissue AGEs, plasma glucose, platelet cytosolic free calcium and blood pressure in fructose-fed WKY rats (30). Similarly, in SHR, NAC treatment lowered tissue AGEs, platelet cytosolic free calcium and blood pressure (23). These data from *in vitro* and *in vivo* experiments demonstrate that NAC lowers aldehydes and AGEs, improves insulin resistance, reduces oxidative stress and lowers cytosolic free calcium.

Cysteine improves bioavailability of NO and endothelial function

One of the major regulators of blood pressure is NO. The enzyme NOS catalyzes the conversion of arginine to NO in the presence of various cofactors. Endothelium-derived NO activates guanylate cyclase to generate cyclic guanosine monophosphate, resulting in VSMC relaxation and vasodilation, thus contributing to the regulation of peripheral blood flow, vascular resistance and blood pressure. eNOS expression and NO formation also result from receptor-mediated stimulation of the IRS/PI3K/Akt/eNOS insulin signalling pathway; thus, their levels are partially regulated by insulin (99-102). NO not only regulates blood pressure but maintains other aspects of vascular health by inhibiting platelet aggregation, VSMC migration and proliferation, and adhesion molecule expression and monocyte adhesion (163). Abnormalities in NO bioavailability may therefore result in inflammation, increased cell proliferation, altered vascular reactivity and elevations in blood pressure.

In humans with essential hypertension, decreased synthesis of endothelium-derived NO partly accounts for both the increase in vascular resistance and the impaired response to endothelium-dependent vasodilation (9-11,164). In animal models of hypertension including SHR, and fructose- and salt-induced hypertensive rats, NO production by resistance arteries is impaired (165-167). In the insulin-resistant state in both humans and animals, abnormalities in insulin-induced NO synthesis via the IRS/PI3K/Akt/eNOS pathway may contribute to altered vascular function and hypertension (102,119,120,168).

Oxidative breakdown of NO gives rise to radicals such as O_2^- , peroxynitrite and peroxynitrous acid (169), not only decreasing NO levels but increasing oxidative stress. An increased production of O_2^- by NADPH oxidase in association with diminished NO generation was found in hypertensive subjects (7). Oxidative stress has been shown to cause 'uncoupling'

of eNOS, disabling the enzyme, producing O_2^- and limiting NO formation (80,170). eNOS contains a free cysteine SH group at its catalytic site, making it susceptible to inactivation by ROS and aldehydes (43). ROS also interfere with signalling via the IRS/PI3K/Akt/eNOS pathway (79), which may result in altered NOS expression and decreased NO production.

As major endogenous antioxidants, cysteine and GSH protect NO, NOS and signalling pathways from the oxidative effects of ROS, thereby preserving NO bioavailability. A study by Ramasamy et al (171) showed that NAC increased eNOS expression and activity in both bovine and human endothelial cells in culture. This effect of NAC was further demonstrated by a study in which addition of NAC to tumour necrosis factor- α -treated human vascular endothelial cells in culture restored eNOS expression and increased NO production (172). Supplementation with 1.44 g/kg bw/day of NAC in drinking water for eight weeks in type 1 diabetic rats increased myocardial eNOS protein expression and plasma nitrate/nitrite, a marker of NO production, concomitantly with a reduction in oxidative stress (173). In this same rat model, 55 mg/day of NAC in drinking water for eight weeks normalized endothelium-dependent vasodilation in the aorta (174). In SHR, NAC treatment improved peroxynitrite-induced impairments in endothelium-dependent vasodilation in the aorta (24). NAC treatment also normalized impaired endothelium-dependent vasodilation in the renal arteries of high salt-treated DSS rats (27). In patients undergoing cardiac catheterization, intra-arterial NAC improved coronary and peripheral endothelium-dependent vasodilation (175), consistent with a preservation of NO function. Contrary to these results with NAC, a methionine-enriched diet impaired endothelium-dependent vasodilation in WKY control rats, consistent with the increase in blood pressure seen in these animals, indicating impaired bioavailability of NO (35). This may be due to incomplete metabolism of methionine to cysteine as illustrated by the increase in homocysteine levels. In SHR, endothelium-dependent vasodilation was also impaired by methionine. However, this was not in agreement with the decrease in blood pressure seen in this model. It was suggested that the hypertensive consequence of methionine supplementation was masked by some other unexplained antihypertensive mechanism in these animals.

Cysteine and glutathione were shown to prevent inhibition of NOS by ebselen, a seleno-organic compound, by providing a competing source of free SH groups (176). In L-NAME-treated Wistar rats, NAC treatment increased NOS activity in the left ventricle and kidney (32). NAC treatment in SHR also increased NOS activity in the left ventricle and kidney, and enhanced eNOS protein expression in the left ventricle (26). In another study of SHR, NAC improved L-NAME-induced impairment in endothelium-dependent vasodilation, suggesting that NAC compensates for the loss of NO (33).

Both cysteine and glutathione react with NO via their SH groups to form SNOs, molecules that are more stable than NO itself. S-nitrosocysteine gives up its NO more readily than S-nitrosoalbumin (49,53), and both serve as part of a transnitrosation system that ferries NO to and from large SH-containing proteins such as albumin (49-51). In the form of S-nitrosoalbumin, NO is even more stable (49,51). Initially, it was thought that SNOs were simple carriers of NO that passively released NO for diffusion into the cell. Subsequently, it

has been shown that NO release may also be initiated by the reaction of SNOs with ions such as copper (177,178), enzymes including thioredoxin reductase (179) and alcohol dehydrogenase (180), or through reaction with membrane thiols (181). Although extending the half-life of NO may indeed be one of the functions and mechanisms of action of SNOs, it has been shown repeatedly that the amount of NO released does not sufficiently account for their total biological activity (52,53,182). It appears that cysteine and glutathione in the form of SNOs likely play a multifaceted role in the complex biology of NO through independent vasoactive properties, transnitrosation reactions, and the balance between their synthesis and degradation (49,52-55).

SNOs have vasoactivity independent of their NO content, and are known to stimulate guanylate cyclase, promote vascular relaxation, reduce platelet aggregation and lower blood pressure (50-53,55,183,184). It has been suggested that S-nitrosocysteine may act via stereoselective membrane-bound receptors (185,186) or through transnitrosation reactions with membrane-bound thiols (181). Scharfstein et al (51) illustrated the potential importance of transnitrosation in the vasoactive biology of SNOs by demonstrating that the vascular sensitivity to S-nitrosoalbumin was enhanced 18-fold by preinfusion of cysteine and threefold by preinfusion of NAC. Another study by Zhang and Hogg (187) also demonstrated this relationship in macrophages. In that study, the presence of cystine (cysteine's redox partner) enhanced cellular uptake of SNO, likely via conversion of cystine to cysteine. This was followed by a transfer of NO from S-nitrosoalbumin to cysteine and subsequent transfer of S-nitrosocysteine to the cytosol via membrane arginine transporters. The stability and rate of decomposition of SNOs may depend on their individual concentrations in circulation, as well as those of other coexisting thiols and SNOs (53). It has been shown that the presence of cysteine decreases the stability of S-nitrosoalbumin, while GSH increases the stability of S-nitrosocysteine, indicating the interactive relationship of these compounds and the potential impact of changes in thiol concentrations on hemodynamic regulation. Because NO is considered to be an intracellular signalling molecule (188), the transnitrosation properties of cysteine and GSH make them potentially important regulators of intracellular functions, such as movement of calcium (67,189), gene transcription and protein expression (190,191), and glucose metabolism (192). In summary, cysteine and glutathione regulate vascular function by protecting NO, NOS and insulin signalling pathways from alteration by ROS. They also act directly on vasculature and participate in the complex biology of NO in the form of SNOs.

Cysteine modulates other vasoactive systems

Another major system involved in blood pressure control is counter-regulatory to the NO pathway. The renin-angiotensin system (RAS) controls vascular tone by converting angiotensin I to the vasoconstrictor AII via ACE. AII acts on membrane receptors in vascular tissue causing vasoconstriction. It also triggers the release of aldosterone to increase renal sodium and water retention.

Although AII infusion has been shown to increase blood pressure in animal models (193), there does not appear to be strong evidence implicating defects in the RAS as a primary

etiological mechanism in essential hypertension in humans. It may be that alterations in RAS occur secondarily as a consequence of oxidative stress (62,72), elevated AGEs (153,194), high insulin concentrations (103,104,195) and decreased NO (196). Once activated, the RAS can contribute to insulin resistance, and increase blood pressure, oxidative stress and cell proliferation (193,197-201).

Cysteine and GSH may contribute to blood pressure control and endothelial function by modulating the RAS. In vitro experiments have demonstrated that exposure of the kidneys, heart and brain to oxidizing agents increases ACE activity (62,72), and NAC and glutathione inhibit this increase when applied to the kidney cortex (62). AGE-RAGE interactions increased AII via an ROS-mediated mechanism in mesangial cells in vitro, and these changes were prevented by NAC treatment (153). It has been suggested that AII may contribute to insulin resistance because it inhibits insulin signalling in aortic VSMCs (197). Taniyama et al (78) showed that AII decreased IRS-1 protein levels in these cells by an ROS-mediated mechanism. This effect was prevented by the addition of NAC. A study by Boesgaard et al (202) showed that intravenous infusion of NAC, 5 mmol/kg bw/h for 3 h in Wistar rats, resulted in a decrease in the pressor effect of an intravenous infusion of angiotensin, and a reduction in plasma and kidney ACE activity. NAC infusion also decreased an earlier isosorbide dinitrate-induced increase in AII in human subjects (202). Because ROS stimulate ACE activity (62) and act as second messengers in many AII-mediated pathways (203), it is likely that cysteine and GSH moderate the vasoconstrictor effects of the RAS mainly via their antioxidant properties. However, an additional mechanism of action has been suggested in another study using VSMCs in vitro. In that study, the addition of NAC and other thiol-containing antioxidants lowered AII binding to AII type 1 receptors (204). Considering that the effect was not seen using nonthiol antioxidants or oxidized NAC, it was suggested that it involved a direct reaction of NAC with the disulfide bonds of the receptor.

In recent years, there has been growing interest in another potential contributor to vascular tone, H₂S. The production of H₂S from cysteine is catalyzed by cystathionine gamma-lyase (CSE) and cystathionine beta-synthase, two vitamin B₆-dependent enzymes involved in the methionine catabolism pathway (205) (Figure 2). At higher concentrations, H₂S causes vascular relaxation in precontracted human mammary arteries (206) and rat aortas (207), and is mediated by the opening of ATP-dependent potassium channels (206). However, at lower concentrations, it causes vascular contraction in these arteries. Infusion of a low concentration of H₂S concurrently with an NO donor results in inhibition of the vasodilator effect of NO (207), suggesting that the contractile action of H₂S is produced via an NO-mediated effect, possibly through the formation of an inactive nitrosothiol. This finding was confirmed in vivo in a rat model (207). This interrelationship suggests there may be cross-talk between H₂S and NO (207,208). Thus, the physiological role for H₂S, although still unclear, may partially involve regulation of NO and vice versa. The role of H₂S in hypertension also remains to be ascertained. Plasma H₂S levels are low in SHR (209) and L-NAME-induced hypertensive rats (208), as well as in children with hypertension (210). In L-NAME-induced

hypertensive rats, H₂S generation is reduced and CSE gene expression is decreased, suggesting that NO may control H₂S generation at the gene level. In these hypertensive animal models in which plasma levels of H₂S are low, administration of an exogenous H₂S donor, NaHS, lowers blood pressure (208). In hypertension, it may be that low NO levels result in reduced expression of CSE with a subsequent decrease in H₂S production, lowering plasma concentrations and increasing vascular contraction. Alternately, it may be that an increase in oxidative stress with an ensuing depletion of cysteine limits H₂S production. Because there is a dearth of research on the role of H₂S in hypertension, these explanations are purely speculative. Certainly, much more research needs to be performed in this area to determine the role of cysteine and/or glutathione in the actions of H₂S on blood pressure.

Cysteine improves renal function

Under normal conditions, the kidneys are key regulators of blood pressure homeostasis; they control blood volume through regulation of sodium excretion and reabsorption. Hypertension itself is a risk factor for developing renal disease (211), as documented by the United States renal disease registry database (212), which lists hypertension as the cause of approximately one-quarter of end-stage renal disease cases from 2000 to 2004. However, changes to kidney function can also cause an increase in blood pressure. This fact is evident in patients with moderate to severe renal disease who experience a very high incidence of hypertension (213). In essential hypertension, the independent, collective or interactive effects of insulin resistance, activation of RAS, decreased NO, or increased oxidative stress and AGEs on the kidneys may contribute to a decrease in kidney function, leading to elevations in blood pressure (27,28,82,83,214-218). If the systemic effects of NAC to normalize or attenuate these alterations lower blood pressure (as previously discussed), this would prevent consequent pressure-induced damage to kidney tissue. Alternatively, if cysteine counteracts the direct pathological effects of these alterations on the kidneys, preserving renal function, this may partially explain its antihypertensive effect. It is probable that cysteine offers renal protection by both means.

Prehypertensive SHR showed increased oxidative stress and inflammation in the kidneys that was attenuated by treatment with NAC (82). Hypertensive SHR and fructose-induced hypertensive rats both demonstrated an increase in kidney AGEs and renal vascular proliferation in conjunction with elevated blood pressures that were reduced by NAC treatment (23,30). In DSS rats, a high-salt diet increased renal oxidative stress and blood pressure, while addition of NAC normalized blood pressure and reduced oxidative stress to preserve kidney function (28). Another study in DSS rats showed that NAC lowered blood pressure, ameliorated renal oxidative stress, improved impaired endothelial-dependent vasodilation in the renal artery and normalized renal function (27). This suggests a mechanism of action by NAC that reduces oxidative stress, thereby preventing breakdown of NO in the kidneys to preserve renal function. This renal-protective effect of NAC was also demonstrated in partially nephrectomized Wistar rats, in which NAC treatment lowered blood pressure, decreased oxidative stress, improved renal function and attenuated the progression to chronic renal failure (219). In a study of renal

microcirculation in SD rats subjected to intravenous vasoconstriction agents, infusion of NAC improved blood flow and lowered vascular resistance by a mechanism other than NO or prostaglandins (220). It is clear that cysteine offers renal protection that may prevent progression to hypertension. As well, it ameliorates changes to the kidneys brought on by hypertension.

CONCLUSIONS

Cysteine and its endogenous storage form, glutathione, are involved in vasoregulatory processes and play a significant role in modulating hypertension. They are important antioxidants that participate in controlling oxidative stress. These thiols improve insulin resistance and lower AGEs, increase bioavailable NO and, in the form of nitrosothiols, participate in the complex biology of NO. They may also be involved in other vasoregulatory systems such as the RAS and H₂S pathway, and provide renal-protective effects. NAC supplementation lowers blood pressure in genetic and salt-, sugar- or L-NAME-induced

rat models of hypertension. NAC has been safely used as a complement to traditional antihypertensive therapies to lower blood pressures in humans. However, there are no studies in humans in which it has been used alone. Therefore, at present, dietary intake of protein may be the appropriate way to achieve an adequate intake of cysteine. Adopting a diet containing ample amounts of protein and antioxidant vitamins, such as the DASH diet – which is rich in vegetables, fruits and low-fat dairy products, low in salt and saturated fat, and includes whole grains, poultry, fish and nuts – may be a safe and realistic means for individuals with hypertension to have an appropriate cysteine intake.

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