Copper deficiency and cardiovascular disease: role of peroxidation, glycation, and nitration[1]

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Abstract: Dietary copper deficiency causes a variety of cardiovascular deficits. Systemic effects include high blood pressure, enhancement of inflammation, anemia, reduced blood clotting, and possibly arteriosclerosis. Effects on specific organs or tissues include weakened structural integrity of the heart and blood vessels, impairment of energy use by the heart, reduced ability of the heart to contract, altered ability of blood vessels to control their diameter and grow, and altered structure and function of circulating blood cells. In some instances, the cause of a defect can be directly attributed to reduced activity of a specific copper-dependent enzyme. However, three nonspecific mechanisms of damage have been implicated in cardiovascular defects of copper deficiency. They are peroxidation, the interaction of oxygen-derived free radicals with lipids and proteins (possibly DNA); glycation, the nonenzymatic glycosylation of proteins; and nitration, the interaction of nitric oxide and its metabolites with peptides and proteins. Though independently these mechanisms present great potential for damage, the possibility that they may interact presents an added reason for concern. Furthermore, the fact that at least two of these mechanisms are associated with diabetes and aging suggests that copper deficiency may exacerbate deficits associated with these two conditions.

Key words: copper, heart, circulation, peroxidation, glycation, nitric oxide.

Résumé : Une carence alimentaire en cuivre entraîne diverses déficiences cardio-vasculaires. Les effets systémiques incluent l'hypertension artérielle, une augmentation de l'inflammation, l'anémie, une réduction de la coagulation sanguine et, possiblement, l'artériosclérose. Les effets sur des tissus ou organes spécifiques se traduisent par un affaiblissement de l'intégrité structurale du cœur et des vaisseaux sanguins, une diminution de la consommation d'énergie par le cœur, une diminution de la capacité du cœur de se contracter, une diminution de la capacité des vaisseaux sanguins de contrôler leur diamètre et de se développer, et une modification de la structure et de la fonction des globules sanguins circulants. Dans certains cas, une anomalie peut être directement attribuée à l'activité réduite d'une enzyme spécifique dépendante du cuivre. Toutefois, trois mécanismes de lésions non spécifiques ont été mis en cause dans les déficiences cardio-vasculaires associées à une carence en cuivre : la peroxydation, ou interaction entre les radicaux libres dérivés de l'oxygène et des lipides et protéines (possiblement l'ADN); la glycation, ou glycosylation non enzymatique des protéines; et la nitration, ou interaction entre le monoxyde d'azote, ses métaboliques et des peptides et protéines. Bien que chacun de ces mécanismes présente un fort potentiel lésionnel, la possibilité qu'ils puissent interagir est inquiétante. De même, le fait qu'au moins deux de ces mécanismes soient associés au diabète et au vieillissement donne à penser qu'une carence en cuivre pourrait exacerber les déficiences associées à ces deux états.

Mots clés : cuivre, cœur, circulation, peroxydation, glycosylation, monoxyde d'azote.

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Cardiovascular effects of dietary copper deficiency

The effects of dietary copper deficiency on the cardiovascular system are numerous and varied (Table 1; Saari and Schuschke 1999). They include gross and microscopically observable structural changes in the heart and blood vessels, functional effects on the heart that include altered energy metabolism and impaired contractile and electrophysiological function, altered circulatory function involving vasoactive, inflammatory, and coagulation deficits and systemic effects that include altered blood pressure, anemia, and hypercholesterolemia.

Mechanisms of effects

Because of the diverse nature of the cardiovascular effects of copper deficiency, a unifying mechanistic view has been difficult to visualize. Four separate categories of cause are discussed below: alteration of copper-dependent enzymes, peroxidation, glycation, and disruption of nitric oxide-dependent processes. Each has strong experimental support, but each, until recently, has been pursued relatively independently. Based on recent findings in copper nutrition, as well as in related fields, that show multiple interrelationships between these four categories, the study of any one cause with no consideration of the others is no longer tenable. The

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Table 1. Cardiovascular effects of dietary copper deficiency.

Findings	Representative references
Heart morphology	
Gross observations	
Heart enlargement (concentric)	(Jalili et al. 1996)
Textural softness	(Shields et al. 1962)
Ventricular aneurisms (some ruptured)	(Viestenz and Klevay 1982)
Microscopic observations	
Connective tissue damage	(Medeiros et al. 1993)
Enlargement, disruption of mitochondria	(Medeiros et al. 1991)
Distorted myocytes	(Borg et al. 1985)
Focal inflammation, hemorrhage, necrosis, fibrosis	(Redman et al. 1988)
Heart function	
Impaired energy metabolism	
Depressed [ATP] and [phosphocreatine]	(Kopp et al. 1983)
Reduced efficiency of oxygen usage	(Prohaska and Heller 1982)
Reduced mitochondrial respiration	(Bode et al. 1992)
Altered mitochondrial ATP synthase	(Matz et al. 1995)
Impaired Na^+/K^+-ATPase	(Huang et al. 1995)
Impaired contractile function	(Prohaska and Heller 1982)
Arrhythmia	(Viestenz and Klevay 1982)
Blood vessel morphology	
Connective tissue damage	
Elastin, collagen (disruption)	(Shields et al. 1962)
Endothelium, smooth muscle, basal lamina (distortion)	(Allen 1990)
Impaired angiogenesis	(Ziche et al. 1982)
Circulatory function	
Altered vasoactivity	
Increased constriction	(Kitano 1980)
Reduced dilation	(Schuschke 1997)
Enhanced inflammation	
Enhanced tissue swelling	(Milanino et al. 1985)
Enhanced leakage of macromolecules	(Schuschke et al. 1989)
Increased number of mast cells	(Schuschke et al. 1994*b*)
Altered coagulation	
Increased clotting time (reduced factor V, factor VIII, von Willebrand factor)	(Lynch and Klevay 1992; Schuschke et al. 1994*a*; Lominadze et al. 1997)
Increased platelet aggregation (increased fibrinogen)	(Lominadze et al. 1996)
Reduced clot dissolution	(Lynch and Klevay 1993)
Altered platelet function (impaired signal transduction)	(Johnson and Dufault 1991; Johnson and Dufault 1993)
Systemic cardiovascular effects	
Altered blood pressure	
Depression in younger animals	(Wu et al. 1984)
Elevation in older animals	(Klevay 1987)
Elevation in response to stressors	(Lukaski et al. 1988)
Anemia	(Cohen et al. 1985)
Hypercholesterolemia	(Allen and Klevay 1978)

remainder of this review will first consider the evidence for the four categories of cause and then discuss a hypothesis that suggests that they are not independent.

Alteration of copper-dependent enzymes

The dependence of specific metalloenzymes upon copper has provided proven and potential explanations of some of the defects. For example, lysyl oxidase is known to form functional cross-links in elastin and collagen (Rucker et al. 1998). Thus, a deficit in its function explains the tissue softness, connective tissue damage, and aneurisms that occur in copper-deficient tissues. Likewise, a functional deficit in copper-dependent cytochrome c oxidase explains some of the impaired efficiency of energy utilization in copper-deficient hearts (Table 1). Because ceruloplasmin is a ferroxidase (Owen, Jr. 1982), its impairment has been associated with impaired iron handling and perhaps the anemia of copper deficiency. Dopamine β-monooxygenase catalyzes the conversion of dopamine to the neurotransmitter norepinephrine (Kaufman and Friedman 1965); with its impairment one may postulate chronotropic and inotropic defects in the heart and vasodilatory defects in the circulation.

Table 2. Evidence for increased peroxidation in copper deficiency.

Finding	Representative references
Reduced activity of antioxidant enzymes	(L'Abbé and Fischer 1984; Lynch and Strain 1989; Prohaska 1991)
Increased susceptibility of tissues to in vitro peroxidation	(Paynter 1980; Fields et al. 1984*b*; Rayssiguier et al. 1993)
Increased lipid and protein peroxidation products in tissues and blood	(Nelson et al. 1992; Chen et al. 1994; Sukalski et al. 1997)
Enhanced breath ethane production	(Saari et al. 1990)
Protection by feeding exogenous antioxidants or by transgenically elevating antioxidant status	(Johnson and Saari 1989; Kang et al. 2000)
Protection by Fe chelation or by reduction of Fe intake	(Fields et al. 1991; Fields et al. 1993)
Increased lung damage with hyperbaric-hyperoxia	(Akers and Saari 1993)
Correlation of defects with peroxidation products	(Saari et al. 1995)

Table 3. Evidence for increased glycation in copper deficiency.

Finding	Representative references
Indirect evidence	
Reduced glucose tolerance	(Keil and Nelson 1934)
Impaired insulin release and binding	(Cohen and Miller 1986; Fields et al. 1983)
Enhancement of defects by fructose relative to those of glucose	(Fields et al. 1984*a*)
Amelioration of effects by food restriction	(Saari et al. 1993; Werman and Bhathena 1993)
Inhibition of effects by aminoguanidine (inhibitor of advanced glycation)	(Saari 1994)
Correlation of defects with glycated Hb	(Saari et al. 1995)
Direct evidence	
Increased early products of glycation (glycated Hb, serum fructosamine)	(Saari and Dahlen 1999)
Increased advanced glycation end-product (serum pentosidine)	(Saari and Dahlen 1999)

Peptidylglycine α-amidating monooxygenase is an enzyme whose activity was recently found to be impaired in dietary copper deficiency (Prohaska et al. 1995). Because it is responsible for amidating and therefore activating a large number of biologically active peptides (Eipper et al. 1992), it is reasonable to postulate that functions associated with these peptides are impaired in copper-deficient subjects.

These and other known copper-dependent enzymes (Prohaska 1990) may be attached to specific functional defects directly associated with their activity. Other defects, however, are not readily associated with the malfunction of a specific copper-dependent enzyme and other mechanisms must be examined.

Peroxidation

Peroxidation involves the production of free radicals that are by-products of both catalyzed and spontaneous metabolic reactions, in particular, those involving oxidation and reduction (Pryor 1973). The free radicals, highly reactive because of unpaired electrons, are then capable of damaging structural and functional characteristics of lipids, proteins, and DNA.

Support for the view that copper deficiency causes peroxidative damage is reviewed in Table 2. The primary observations that support an oxidative theory of copper deficiency are the reduction of activities of several copper-dependent antioxidant enzymes, superoxide dismutase, ceruloplasmin and cytochrome c oxidase, and possibly non-copper-dependent enzymes such as catalase. These findings have been complemented by observations of increased peroxidative products in blood and tissues, by increased susceptibility to oxidative damage in copper-deficient animals, by correlation of severity of the defects with extent of peroxidation, and by amelioration of defects such as cardiac enlargement and anemia by treatment with antioxidants.

Glycation

Glycation is the nonenzymatic binding of the acyclic form of a sugar to proteins, preferably to lysine and hydroxylysine residues, that compromises protein structure and function and can lead to cross-linking and degradation of the protein (Reiser 1991). Glycation is elevated in conditions, in which blood sugar is elevated such as diabetes mellitus and aging.

Copper deficiency has long been associated with altered carbohydrate metabolism. Indirect and direct evidence that glycation contributes to the defects of copper deficiency is summarized in Table 3. The earliest observation was of a reduced glucose tolerance in copper-deficient animals. Subsequent studies have indicated that altered insulin metabolism plays a role because both pancreatic insulin release and insulin's effects on target organs are impaired. Amelioration of defects of copper deficiency by food restriction, which lowers blood glucose, enhancement of defects by fructose, a better glycator than glucose, and correlation of defects with extent of glycation provide indirect support for glycation as a cause of defects of copper deficiency. Although elevation of blood glucose is not commonly seen in fasting copper-deficient animals, the hypothesis that glucose is chronically elevated, and further, that it causes actual damage in copper deficiency, is supported by the observation of increases in both early and advanced glycation end-products.

Table 4. Nitric oxide and Cu deficiency.

Findings	Representative references
Blood vessels	
Endothelium-dependent responses are impaired in aorta and microcirculation	(Saari 1992; Schuschke et al. 1992)
Superoxide contributes to impaired responses	(Schuschke et al. 1995; Lynch et al. 1997)
Blood peroxynitrite is elevated	(Schuschke et al. 2000)
Impaired Ca^{2+} handling contributes to impaired responses	(Schuschke et al. 2000)
eNOS protein is unaffected	(Schuschke et al. 2000)
Heart	
Nitric oxide metabolites are elevated in heart (and urine)	(Saari and Dahlen 1998)
iNOS protein is elevated in the heart	(Saari and Bode 1999)
iNOS induction is exaggerated by copper deficiency in presence of iNOS inhibition	(Saari and Bode 1999)

Altered nitric oxide metabolism

Nitric oxide may be produced by one of three isoforms of nitric oxide synthase (Nathan and Xie 1994). Endothelial nitric oxide synthase [eNOS or NOS (3)] and neuronal nitric oxide synthase [nNOS or NOS (1)] are constitutive, dependent on elevation of intracellular calcium for activation, and assist in regulation of cellular responsiveness to hormones, neurotransmitters, and growth factors (Ignarro 1990; Jaffrey and Snyder 1995). The third isoform [iNOS or NOS (2)] is found in macrophages and parenchymal cells such as cardiac myocytes, is independent of calcium, and may be induced by such stimuli as cytokines, inflammatory mediators, and notably oxygen-derived free radicals (Rubbo et al. 1996).

The alteration of nitric oxide-dependent processes by copper deficiency is outlined in Table 4.

Circulatory effects of copper deficiency

Alteration of nitric oxide-mediated events by copper deficiency was first observed in the circulation. In both large and small blood vessels, endothelium-dependent vasodilation was shown to be reduced. Further study supported the view that superoxide, expected to be elevated in copper deficiency because of reduced superoxide dismutase activity, interacts with nitric oxide, reducing its vasodilatory effect on vascular smooth muscle. Recent findings indicate that peroxynitrite, the product of this interaction, interferes with endothelial calcium mobilization, reducing activation of eNOS and impairing nitric oxide-mediated vasodilation by a second mechanism.

Cardiac effects of copper deficiency

Recent studies have revealed that copper deficiency also affects nitric oxide metabolism in the heart. We expect that, when studied, the coronary circulation will be affected in a similar way as other blood vessels with respect to endothelial nitric oxide, but the heart as a whole shows different responses to copper deficiency than does the circulation. Whereas in the microcirclation eNOS expression is not elevated and nitric oxide mediated responses are depressed, in the whole heart total nitric oxide production is elevated. This has been associated with an elevation of cardiac iNOS protein. Although the site of elevated iNOS in copper deficiency is unknown, in other studies, iNOS has been found in both cardiac myocytes (Schulz et al. 1992) and invading macrophages of sick hearts (Wildhirt et al. 1995).

A new hypothesis suggesting synergism and interaction of mechanisms

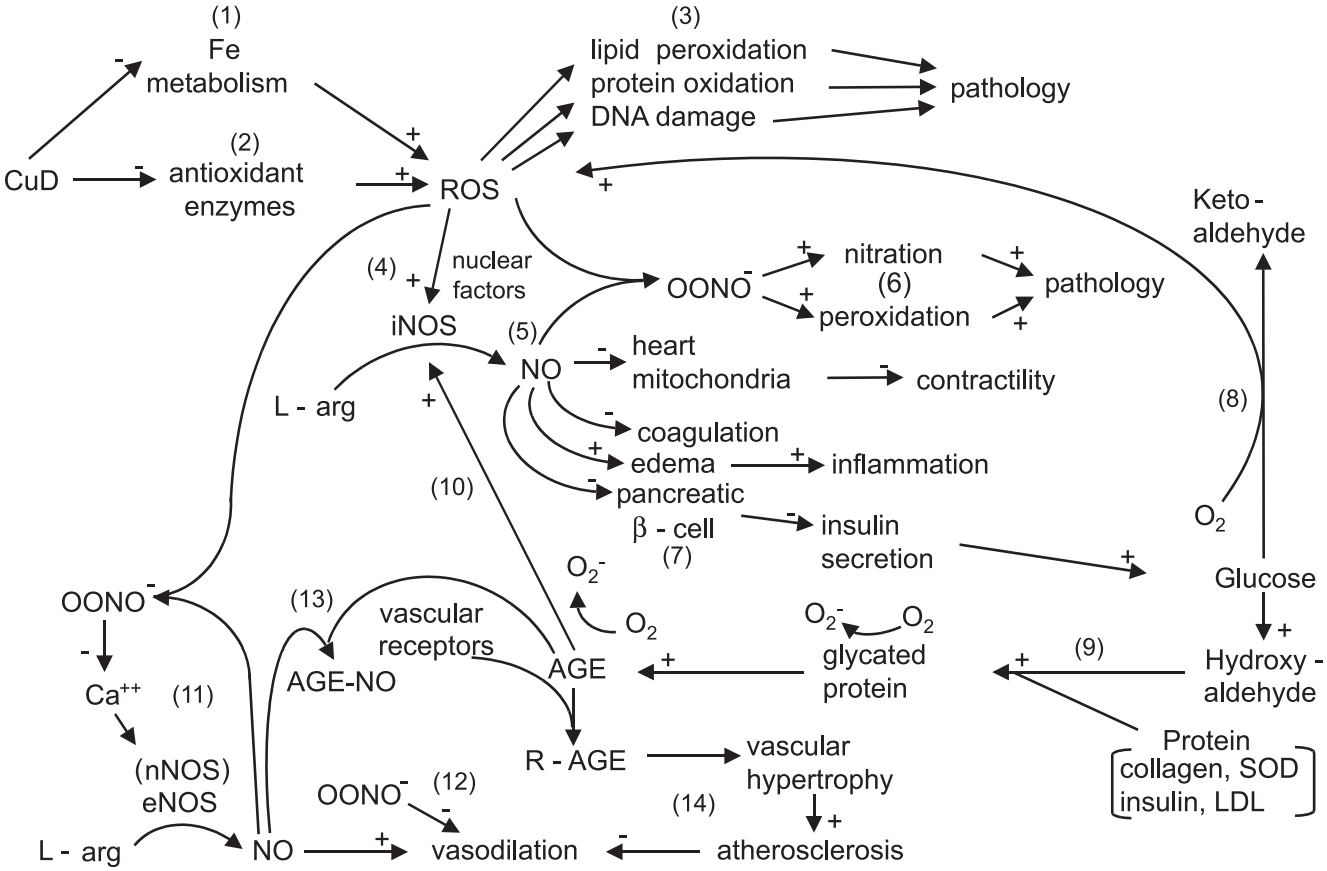
Prior hypotheses and suggestions for causes of the cardiovascular effects of copper deficiency have been relatively straightforward: *i*) copper deficiency depresses the function of a copper enzyme, which impairs related morphology or physiology, *ii*) copper deficiency depresses functions of antioxidant enzymes, allowing accumulation of oxygen-derived free radicals, which initiate lipid, protein, and DNA damage, causing impairment of their associated functions, *iii*) copper deficiency impairs carbohydrate metabolism, allowing the accumulation of sugar and encouraging protein glycation and the ultimate degradation of their structure and function, and *iv*) copper deficiency interferes with enzymes related to nitric oxide-mediated signal transduction, impairing or exaggerating associated functions.

In our pursuit of the above lines of research, it became increasingly clear that some of the above mechanisms of copper deficiency could not be dissociated from one another, e.g., elevation of peroxidation products and glycation products were found in the same experiment and ameliorative or exacerbating treatments affected both. Some of the mechanisms clearly interacted with one another, e.g., superoxide interfered with nitric oxide-mediated signal transduction. A review of the literature on diabetes, aging, peroxidation, glycation, and nitration has shed some light on what we are beginning to see in copper deficiency and has resulted in the formulation of a hypothesis that is represented by Fig. 1.

The upper portion of Fig. 1 [pathways (1)–(3)] represents the effects of copper deficiency that are reasonably well established (see Table 2). Copper deficiency (CuD) causes impairment of iron metabolism (1) that may contribute to iron-catalyzed production of reactive oxygen species (ROS) (Fields et al. 1991, 1993). Copper deficiency is known to cause reduced activity of copper-dependent antioxidant enzymes (2), which allows the build-up of reactive oxygen species produced by metabolism. The increased reactive oxygen species then lead to molecular damage (3) and subsequent pathology.

The middle portion of Fig. 1 [pathways (4)–(10)] suggests how copper deficiency, largely through production of reactive oxygen species, may increase the production of nitric oxide (NO) and impair cardiovascular function. This suggestion hinges on the known ability of ROS to induce expres-

Fig. 1. Postulated relationships between copper deficiency, peroxidation, glycation and nitric oxide-mediated responses. Numbers in parentheses are points of reference to pathways discussed in the text. Plus and minus signs indicate whether a given effect is enhanced or reduced by the preceding stressor. Abbreviations: CuD, copper-deficient state; Fe, iron; ROS, reactive oxygen species; iNOS, inducible nitric oxide synthase [also, NOS (2)]; NO, nitric oxide; $OONO^-$, peroxynitrite; O_2, oxygen; O_2^-, superoxide; Ca^{2+}, free calcium; AGE, advanced glycation end-product; AGE-NO, adduct of AGE and NO; R-AGE, receptor-bound AGE; nNOS, neuronal nitric oxide synthase [also, NOS (1)]; eNOS, endothelial nitric oxide synthase [also, NOS (3)]; L-arg, L-arginine; SOD, superoxide dismutase; LDL, low density lipoprotein.



sion of iNOS via activation of nuclear transcription (4) (Rubbo et al. 1996) and that iNOS expression is enhanced in copper-deficient hearts (Saari and Bode 1999). From yet other experimental models, nitric oxide is known to impair a variety of functions that we know are disrupted in copper deficiency (Table 1). For example (5), elevated nitric oxide is known to impair heart contractile (Balligand et al. 1993) and mitochondrial function (Wolin et al. 1997), to reduce coagulation (Wu and Thiagarajan 1996), to enhance edema formation (Mayhan 1994) and, at high concentrations, to cause pancreatic β-cell damage and reduce insulin production (Corbett et al. 1993). The concomitant increases in superoxide and nitric oxide may lead to an increase in peroxynitrite ($OONO^-$), presenting the additional question of whether peroxynitrite causes the pathologies attributed to nitric oxide either by contributing to peroxidation or nitration (6) (Beckman and Koppenol 1996).

Perhaps the most insidious of the above pathologies associated with elevated nitric oxide production is the effect on the pancreas (7), because it contributes to several forms of positive feedback in the pathological scheme. First, the elevation of blood glucose may glycate proteins (9) that are already compromised by copper deficiency. Two notable proteins that are susceptible to glycation are superoxide dismutase (Oda et al. 1994) and insulin (Dolhofer and Weiland 1979), the impairment of which would lead to obvious positive feedback on peroxidation and glycation, respectively. Second, advanced glycation end-products have been shown to enhance the expression of iNOS (10) (Rojas et al. 1996). And finally, glucose may autooxidize (8) (Hunt et al. 1988), contributing to the already elevated pool of reactive oxygen species. Glycated proteins may also autooxidize (Monboisse et al. 1990; Yim et al. 1995) and exaggerate this positive feedback effect.

The lower portion of Fig. 1 [pathways (11)–(14)] suggests how copper deficiency, by virtue of its elevation of peroxidation and glycation, contributes to reduced vasodilation of blood vessels through interaction with nitric oxide-dependent processes. Part of the scheme (11) has direct support from experiments on copper deficiency, that is, the previously described interaction of endothelial nitric oxide with superoxide, peroxynitrite production, and interference with calcium mobilization, all of which ultimately reduce nitric oxide-dependent vasodilation. Studies unrelated to copper nutrition have shown that peroxynitrite can impair vasodilation (12) (Villa et al. 1994), that advanced glycation

end-products (AGE) can scavenge NO (13) (Bucala 1996), and that AGEs interacting with vascular receptors for AGE can lead to vascular pathology, atherosclerosis and impairment of vasodilation (14) (Schmidt et al. 1994; Vlassara and Bucala 1996).

Conclusions

Evidence has been presented that dietary copper deficiency can lead to extensive cardiac, circulatory, and systemic effects on the cardiovascular system. These effects have been attributed to deficits in Cu-dependent enzymes, peroxidation, glycation, and alteration of nitric oxide-dependent processes. Recent findings in copper-deficient animals coupled with a review of a parallel literature in aging and diabetes suggest that these four mechanisms are not independent. This realization has resulted in an interactive hypothesis that gives direction to future studies on copper deficiency.

A corollary conclusion resulting from the above findings is that, because peroxidation and glycation have long been associated with both aging and diabetes and may be considered as mechanisms of damage in those two conditions, adequate copper nutriture may be essential in both the elderly and diabetics to prevent enhancement of pathology. The more recent association of nitric oxide with pancreatic dysfunction and of disturbed nitric oxide-dependent processes in diabetes further emphasize the interactions postulated above as they apply to a health setting.

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