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Captopril has no significant scavenging antioxidant activity in human plasma *in vitro* or *in vivo*

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1 Captopril has been reported to possess hydroxyl radical (OH·) and hypochlorous acid (HOCl) scavenging effects, which could contribute to its therapeutic activity in the clinical setting.
2 The objective of the present study was to determine whether therapeutically achievable captopril concentrations could augment antioxidant properties of human plasma and protect it against OH·- and HOCl-driven oxidant injury *in vitro*. Possible drug influences on systemic oxidative stress status *in vivo* were also investigated in subjects taking 50 mg captopril orally by measuring plasma and red blood cell peroxidation, as well as plasma protein thiols.
3 The results show that captopril is incapable of enhancing antioxidant properties of human plasma, of protecting it against specific oxidative attack and of decreasing systemic oxidant load *in vivo*.
4 The present data, therefore, do not support the contention of a beneficial action of captopril through systemic antiradical-antioxidant effects in human beings.

Keywords captopril antioxidant oxidative stress hydroxyl radical hypochlorous acid

Introduction

Experimental investigations have suggested that captopril may exert some beneficial effects in the clinical setting as a result of its thiol group-related antioxidant properties [1–3]. In this regard, captopril has been shown to scavenge efficiently only hydroxyl radical (OH·) and hypochlorous acid (HOCl) [3]. It must be noted, however, that high captopril concentrations and aqueous buffers in the absence of biomolecules present in the blood environment *in vivo* have usually been used to study captopril antioxidant effects *in vitro*. These biomolecules, such as albumin, can readily react with OH· and HOCl [4, 5]. Thus, it seems relevant for an appropriate evaluation of captopril antioxidant activity to investigate whether the drug is capable of enhancing antioxidant properties of human plasma, as well as of protecting endogenous biomolecules against oxidant aggression and of decreasing systemic oxidant burden in humans. These issues have specifically been addressed in the present paper.

Methods

Captopril added to human plasma

Reagents were from Sigma Aldrich s.r.l., Milano, Italy. Heparinized plasma was obtained from seven male healthy adults (35–45 years old).

We at first investigated whether captopril, added to human plasma at therapeutically relevant concentrations, could augment antioxidant properties of plasma itself against OH·- and HOCl-induced oxidant injury.

Regarding OH·, it is known that this oxygen radical is also generated via Fenton chemistry reactions in the presence of EDTA-complexed iron, H_2O_2 and ascorbate as the reducing agent [3–7]. Once produced, OH· can damage oxidatively the sugar deoxyribose (DOR), an integral component of DNA [4, 5], producing aldehydic substances capable of reacting with thiobarbituric acid (TBA) under acidic conditions [3–7]. Biological or pharmacological compounds able to scavenge efficiently OH· can counteract OH·-dependent DOR oxidation and TBA-reactants (TBA-R) formation [3–7]. Reaction mixtures (1.0 ml) contained 10 mmol l^{-1} potassium

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phosphate buffer, pH 7.4, 2.8 mmol l^{-1} DOR, 10 μmol l^{-1} $FeCl_3$ (pre-mixed with 50 μmol l^{-1} EDTA), 0.7 mmol l^{-1} H_2O_2 and 50 μmol l^{-1} ascorbic acid, with and without 50 μl of plasma containing or not 10, 50 and 100 μmol l^{-1} captopril (final drug concentrations were therefore 0.5, 2.5 and 5 μmol l^{-1}). After 30 min incubation at 37° C, 1.0 ml^{-1} of 2.5% trichloroacetic acid (TCA), 1.0 ml^{-1} of 0.6% TBA aqueous solution and 10 μl of 5% butylated hydroxytoluene (BHT) in ethanol were added, followed by 15 min heating at 95° C. After cooling, the red chromogen, yield of OH·-induced DOR oxidation [3–7], was extracted with n-butanol and read spectrophotometrically at 532 nm against an appropriate blank. Results were calculated as nmol TBA-R/μmol DOR, using a molar extinction coefficient of 154, 000.

Antioxidant effects of control and captopril-containing plasma samples against HOCl were evaluated testing their capability to inhibit HOCl-induced 5-thio-2-nitrobenzoic (TNB) oxidation [8]. In this context, it is known that the highest oxidant capacity of HOCl is explicated against sulphhydryl groups [5, 8, 9]; the thiol compound TNB, therefore, is readily oxidized by HOCl, this phenomenon being inhibitable by HOCl scavengers [8]. TNB was prepared through reduction of 5, 5′-dithiobis(2-nitrobenzoic acid) (DTNB) with 2-mercaptoethanol [8], and its concentrations were calculated using a molar extinction coefficient of 13, 600 at 412 nm [8]. Reaction mixtures (1.0 ml) contained 10 mmol l^{-1} potassium phosphate buffer, pH 7.4, 80 μmol l^{-1} TNB, 50 μl of plasma with or without captopril and 33 μmol l^{-1} HOCl. After 15 min incubation at 25° C, absorbance values at 412 (A_{412}) nm were read spectrophotometrically, using appropriate plasma- and captopril-containing blanks. The concentrations of NaOCl-derived HOCl were calculated by the TNB oxidation method of Thomas *et al.* [8], or using a molar extinction coefficient of 100 at 235 nm [4, 7].

Captopril present in the blood after drug administration in humans

In a second set of experiments, we investigated whether captopril, once present in the blood environment after drug administration in humans, could lower systemic oxidant load *in vivo* and protect plasma against OH·-driven peroxidation and HOCl-induced oxidation of protein thiols (P-SH). Five healthy subjects (31–43 years old) of our institutional medical and technical staff were studied twice at a 48 h interval. In the first day, after plasma and erythrocyte collection for specific biochemical tests in basal conditions, placebo tablets were administered orally in the morning in fasting conditions and, after 1 h, blood was collected again to repeat the assays. In the second day, the same experimental approach was followed, except that 50 mg captopril tablets (Capoten, Bristol-Myers Squibb S.p.A., Roma, Italy) were administered by mouth; after 1 h, plasma free captopril levels were also measured essentially as previously reported [10]. In this regard, it must be noted that plasma peak concentrations of free captopril are reached after 1.0 h from drug intake in humans, followed by their rapid and marked decrement [10, 11].

Systemic oxidative stress status before and after captopril intake was evaluated assessing erythrocyte and plasma peroxidation, as well as plasma P-SH levels. Erythrocyte peroxidation was assayed basically as reported by Clot *et al.* [12], after reaction of 1.0 ml^{-1} of the protein-free supernatant resulting from 20% TCA-treated erythrocytes with 1.0 ml^{-1} of 0.6% TBA aqueous solution. After 30 min heating in the presence of 15 μl of 5% BHT in ethanol, the chromogen was extracted with n-butanol and read at 532 nm against an appropriate blank. Notably, this assay gives results strictly related to those of malonildialdehyde measurement by a direct h.p.l.c. method [12], and it has been pointed out as a valuable index of oxidative stress *in vivo* [12]. Plasma lipid peroxidation was studied through the assay of TBA-R, fluorescent damage products of lipid peroxidation (FDPL) and conjugated dienes (CD). For TBA-R, 0.5 ml^{-1} aliquots of plasma were added to 0.5 ml^{-1} of 2.5% TCA, 20 μl of 5% BHT in ethanol, 0.1 ml^{-1} of 8.1% sodium dodecyl sulphate and 0.5 ml^{-1} of 0.6% TBA aqueous solution, followed by 30 min heating at 95° C. The chromogen was then extracted with n-butanol and read at 532 nm against an appropriate blank. FDPL were assessed according to Dillard & Tappel [13], with some modifications. Plasma lipids were extracted with 6.0 ml^{-1} of chloroform/methanol (2 : 1, vol/vol), followed by 2 min vortex mixing and bi-distilled water addition. After a brief centrifugation, the chloroform phase was dried under a flow of argon gas. The residue was resuspended in cyclohexane and subjected to fluorometric study at 360/430 nm excitation/emission, as well as to ultraviolet spectrophotometric study at 233 nm to assay CD [14]. Plasma P-SH represent adequate indicators of oxidative damage *in vivo* [4, 5, 15, 16], since P-SH are oxidized and consumed by oxidant species [4, 5, 15, 16]. P-SH were measured spectrophotometrically at 412 nm reacting 50 μl of plasma with 0.25 mmol l^{-1} DTNB (Ellman's reagent) in 0.2 mol l^{-1} potassium phosphate buffer, pH 8.5, plus 2.0 mmol l^{-1} EDTA [16]. Oxidant-stimulated plasma peroxidation was induced using a copper/H_2O_2 OH· generating system [4, 5], since copper is more active than iron at triggering peroxidation of plasma lipids [17]. The prooxidant system contained 80 μmol l^{-1} $CuCl_2$ and 0.55 mmol l^{-1} H_2O_2, with incubation for 180 min at 37° C; TBA-R, FDPL and CD were then measured, as described above. Regarding HOCl-mediated plasma oxidant damage, 105 μmol l^{-1} HOCl were added to plasma samples obtained before and after drug intake, followed by 30 min incubation at 25° C. Since HOCl oxidizes preferentially thiol groups [5, 8, 9], the loss of plasma P-SH induced by HOCl was measured spectrophotometrically at 412 nm as described above.

Statistics

Data were calculated as means ± s.d. The effects of captopril added to plasma were evaluated by the

one-way analysis of variance plus Student-Newman-Keuls test [18]. In the experiments performed with blood of subjects taking placebo or captopril tablets, results were analyzed by Student's *t*-test for paired or unpaired data, where appropriate [18]. $P<0.05$ was regarded as statistically significant [18].

Results

As shown in Table 1, captopril added to human plasma was incapable of enhancing antioxidant properties of plasma itself against either OH· or HOCl.

Plasma drug concentrations were 1.54 ± 0.38 μmol l^{-1} after 1.0 h from 50 mg captopril tablet intake. At this time, systolic and diastolic blood pressure values fell from 127 ± 5 and 81 ± 5.5 mmHg to 113 ± 4.5 and 69 ± 7.2 mmHg, respectively (both $P<0.01$, paired Student's *t*-test). Since the biochemical data observed in the first 'placebo day' were similar to those of the second 'drug day', and neither placebo nor captopril affected baseline biochemical data, we will report only the results obtained with captopril administration. Notably, the levels of plasma peroxidation were not significantly different before and after captopril intake (0.85 ± 0.1 *vs* 0.87 ± 0.09 nmol TBA-R ml^{-1} of plasma for TBA-R, 24 ± 3.1 *vs* 24.5 ± 3.5 units of relative fluorescence ml^{-1} of plasma for FDPL, and 1.65 ± 0.2 *vs* 1.7 ± 0.25 absorbance units at 233 nm ml^{-1} of plasma for CD), and neither were those of erythrocyte peroxidation (0.56 ± 0.13 *vs* 0.58 ± 0.12 nmol TBA-R ml^{-1} of packed cells) and of plasma P-SH (432.5 ± 27 *vs* 428 ± 25.5 nmol ml^{-1} of plasma). Captopril intake, therefore, did not result in decreased systemic oxidative burden *in vivo* at drug peak concentrations. Table 2 shows that the copper/H_2O_2 oxidant system induced a significant increase of plasma TBA-R ($P<0.01$), FDPL ($P<0.01$) and CD ($P<0.025$) with respect to the basal values; comparable lipid peroxidation levels, however, were observed in the pre- and post-captopril period plasma samples ($P=$NS; Table 2). When plasma was challenged with HOCl, its P-SH content underwent a significant decrement ($P<0.01$), which was about -17 and -18% in the plasma obtained before and after captopril intake, respectively ($P=$NS; Table 2).

Table 1 Effects of human plasma with and without captopril supplementation on OH·- and HOCl-induced oxidative damage

OH·-induced DOR oxidation	
	nmol TBA-R μmol^{-1} DOR
Control	9.1 ± 0.75
Control plus 50 μl of plasma	3.9 ± 0.55*
Control plus 50 μl of plasma and captopril:	
0.5 μmol l^{-1} captopril	3.8 ± 0.6*‡
2.5 μmol l^{-1} captopril	4.0 ± 0.45*‡
5 μmol l^{-1} captopril	3.95 ± 0.5*‡
HOCl-induced TNB oxidation	
	A_{412}
Control (80 μmol l^{-1} TNB)	1.075 ± 0.020
Control plus 33 μmol l^{-1} HOCl	0.182 ± 0.011*
Control plus HOCl and 50 μl of plasma	0.477 ± 0.031*†
Control plus HOCl and 50 μl of plasma with captopril:	
0.5 μmol l^{-1} captopril	0.490 ± 0.028*†‡
2.5 μmol l^{-1} captopril	0.472 ± 0.025*†‡
5 μmol l^{-1} captopril	0.483 ± 0.033*†‡

Means ± s.d. of seven experiments performed on plasma samples of seven healthy subjects. See Methods section for further explanations. *$P<0.05$ *vs* control, †$P<0.05$ *vs* control plus HOCl, and ‡$P=$NS *vs* plasma without captopril (one way analysis of variance followed by Student-Newman-Keuls test).

Discussion

The present study shows that captopril, at therapeutic concentrations, does not enhance antioxidant properties of human plasma nor lower systemic oxidant load *in vivo* or protect endogenous biomolecules against oxidative damage. A possible concern potentially arising from our data may be related to the physiological relevance of the oxidants OH· and HOCl used and of their concentrations. It could be indeed speculated that some captopril antioxidant effects might have been seen with milder degrees of oxidant attack. The aforementioned oxidants have been selected in light of the unambiguous experimental evidence that captopril can react with and scavenge only OH· and HOCl [3], which are the most toxic and relevant oxidizing species [4, 5]. It should be noted that the concentrations of HOCl used in the study appear effectively 'physiologically' relevant, considering that activated neutrophils can generate even more than 100 μmol l^{-1} HOCl [19]. Regarding OH·, instead, its *in vivo* levels are to date unknown. In our first set of experiments, however, the concentration of OH·-generating prooxidants was half that usually used *in vitro* to assess OH· scavenging capacity of various drugs [3–7], while in the second set oxidant concentrations were the lowest possible to induce a clearly detectable plasma peroxidation. On the other hand, in the second set of experiments putative antioxidant effects of captopril in humans were evaluated also in the absence of any 'artificial' oxidative challenge. In fact, in this specific approach plasma and erythrocyte oxidant load was really physiological, because it was simply related to the intrinsic radical generation, as opposed to the endogenous antioxidants, of human beings before and after captopril intake. In any event, basic conceptual aspects do not point to an effective scavenging antioxidant activity of captopril in the blood environment *in vivo*, whatever could be the degree of oxidant load

Table 2 Effects of 50 mg captopril intake on oxidant-induced plasma peroxidation and P-SH oxidation

	Pre-captopril		*Post-captopril*	
	Basal	Cu^{++}/H_2O_2	*Basal*	Cu^{++}/H_2O_2
TBA-R	0.85± 0.1	1.29±† 0.17	0.87±‡ 0.09	1.32±†‡ 0.18
FDPL	24± 3.1	33.5±† 4.3	24.5±‡ 3.5	34.7±†‡ 3.9
CD	1.65± 0.2	2.15±* 0.3	1.7±‡ 0.25	2.3±*‡ 0.4
	Basal	*HOCl*	*Basal*	*HOCl*
P-SH	432.5± 27	358±† 20.7	428±‡ 25.5	351.8±†‡ 23

Plasma and erythrocyte thiobarbituric acid reactants (TBA-R) are expressed as nmol TBA-R ml^{-1} of plasma and nmol TBA-R ml^{-1} of packed cells, respectively. Plasma fluorescent damage products of lipid peroxidation (FDPL) and conjugated dienes (CD) are given as units of relative fluorescence ml^{-1} of plasma and absorbance units at 233 nm ml^{-1} of plasma, respectively. Plasma P-SH are expressed as nmol P-SH ml^{-1} of plasma. See Methods section for further explanations. Means ± s.d. of five experiments performed on different plasma samples of five subjects before and 1.0 h after the intake of 50 mg captopril tablets. $*P < 0.025$, and $\dagger P < 0.01$ *vs* Basal (unpaired Student's *t*-test); ‡P = NS *vs* the respective values of the pre-captopril period (paired Student's *t*-test).

operative in various conditions. Blood contains indeed strong antioxidant defences, resulting in a high antioxidant capacity [4, 5]. For example, albumin reaches mean concentrations of about 650 μmol l^{-1} in human plasma, whereas peak plasma levels of captopril are 0.5–1.5 μmol l^{-1} in humans after administration of usual drug doses of 25–50 mg [10, 11]. Moreover, albumin readily reacts with HOCl [4, 5, 9], and is characterized by a second order rate constant for the reaction with OH· of 2.3×10^{10} mol^{-1} s^{-1} [5], which is near sevenfold higher than that of captopril, i.e. 3.6×10^{9} mol^{-1} s^{-1} [3]. Thus, OH· and HOCl react with 650 μmol l^{-1} albumin and not with 0.5–1.5 μmol l^{-1} captopril (this was exactly the case in our second experimental set). Similar considerations may be done for other physiological plasma antioxidants, such as glucose, ascorbate and uric acid, which reach mean concentrations of 4500, 70 and 300 μmol l^{-1}, respectively [5]. In this regard, it is noteworthy that plasma uric acid concentrations may be increased in hypertensive patients with or without congestive heart failure, a typical clinical setting of captopril use in humans [20]. The aforementioned biochemical aspects appear relevant also in the case of our first set of experiments, where all plasma antioxidants were somewhat 'depleted' because of the 20-fold dilution of the 50 μl plasma aliquots in 1.0 ml^{-1} reaction mixtures. In these conditions, considering only albumin, ascorbate, glucose and uric acid, it may be calculated that their final concentrations in experimental tubes were about 33, 3.5, 225 and 15 μmol l^{-1} , respectively, for an 'antioxidant sum' of 276 μmol l^{-1}. Captopril, at 0.5–5 μmol l^{-1} concentrations, failed to enhance plasma antioxidant properties against oxidative damage; indeed, oxidant species reacted preferentially with the endogenous antioxidants, which, although so diluted, had higher concentrations than captopril itself. Moreover, plasma is endowed with several antioxidant defences, which specifically work in a synergic way to cope with oxidant injury. For example, transferrin binds-inactivates catalytic iron, while albumin scavenges OH· arising from iron-mediated reactions [4, 5]. This synergism of action appears relevant and amplifies the endogenous antioxidant capacity, besides a mere problem of antioxidant concentration. Captopril is characterized not only by very low therapeutic levels [10, 11], but is also a poor thiol donor [21], which does not contribute to plasma antioxidant properties. Thus, for concentration and kinetic problems, captopril is not an effective scavenging antioxidant in the blood environment.

Experimental studies may suggest that captopril could result in some 'indirect-type' antioxidant effects *in vivo*, potentially due to decreased oxidant generation by neutrophils [22] and enhanced production of prostacyclin [23], which has antioxidant properties [24]. However, our data show that indicators of systemic oxidative stress, such as plasma lipoperoxidation and P-SH, as well as oxidant-driven plasma peroxidation and P-SH oxidation, are unaffected by captopril intake at peak drug concentrations, pointing to no significant antioxidant effect of captopril *in vivo*. Moreover, captopril intake does not affect erythrocyte peroxidation, suggesting that also cell systems may not benefit from a putative drug antioxidant activity *in vivo*. In this context, it has been shown that the concentrations of free captopril remain virtually unchanged after drug incubation with erythrocytes [25], indicating that the

thiol group of captopril is not given to cell systems to enrich their antioxidant potential.

The antioxidant ineffectiveness of captopril emerged in our study may be partly in conflict with some experimental evidences showing drug antioxidant effects against human low density lipoprotein (LDL) oxidation *ex vivo*. Indeed, it has been reported that captopril is able to counteract copper-driven LDL oxidation *in vitro*, this effect being significant at 10 μmol l^{-1} drug concentration [26]. In another similar study, however, captopril has been shown to be ineffective at inhibiting copper-mediated human LDL oxidation at about 70 μmol l^{-1} drug concentration, a marked antioxidant activity being evident only at 460 μmol l^{-1} [27]. Therefore, in order to be inhibitory against LDL oxidation, captopril has to achieve relatively high concentrations, which are not therapeutically relevant [10, 11]. Moreover, the model of LDL oxidation *in vitro* is somehow artificial, because of the absence of various key water-soluble plasma antioxidants, such as albumin, ascorbate and urate, which reach far higher concentrations than those of captopril and can inhibit LDL oxidation [28]. Remarkably, in our study all plasma antioxidants were instead present with therapeutic drug concentrations. Different experimental models may so explain the discrepancies arising from different studies. It is also worth emphasizing that captopril is bound in human plasma primarily to albumin (and not to LDL) through covalent disulphide bonds [25], further suggesting that a direct drug antioxidant activity towards LDL is not feasible *in vivo*.

To date, little is known about captopril antioxidant activity in humans. In this regard, Sobotka *et al.* [29] reported that captopril, at mean doses of about 50 mg day^{-1}, reduced the output of pentane (a lipid peroxidation index) in patients with congestive heart failure, apparently showing drug antioxidant effects *in vivo*. These authors, however, have measured breath pentane via a gas chromatographic technique, using a Chromosorb column and a flame ionization detector [29]. This method has been shown to be subjected to some pitfalls and not specific for pentane, thus appearing poorly reliable [30, 31]. Even if further studies may be advisable to investigate whether captopril, alone or in association with other drugs usually used in patients with congestive heart failure, could improve various oxidative stress indices in this clinical setting, our data indicate that systemic antiradical-antioxidant effects of captopril are not feasible in humans.

References

1 Chopra M, Scott N, McMurray J, McLay J, Bridges A, Smith A, Belch JJ. Captopril: a free radical scavenger. *Br J Clin Pharmacol* 1989; **27**: 396–399.

2 Bagchi D, Prasad R, Das DK. Direct scavenging of free radicals by captopril, an angiotensin converting enzyme inhibitor. *Biochem Biophys Res Commun* 1989; **158**: 52–57.

3 Aruoma OI, Akanmu D, Cecchini R, Halliwell B. Evaluation of the ability of the angiotensin-converting enzyme inhibitor captopril to scavenge reactive oxygen species. *Chem-Biol Interact* 1991; **77**: 303–314.

4 Halliwell B. How to characterize a biological antioxidant. *Free Rad Res Commun* 1990; **9**: 1–32.

5 Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. Oxford: Clarendon Press, 1989.

6 Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: A simple 'test-tube' assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem* 1987; 165: 215–219.

7 Lapenna D, de Gioia S, Mezzetti A, Grossi L., Festi D, Marzio L, Cuccurullo F. H_2-receptor antagonists are scavengers of oxygen radicals. *Eur J Clin Invest* 1994; 24: 476–481.

8 Thomas EL, Grisham MB, Jefferson MM. Preparation and characterization of chloramines. *Methods Enzymol* 1986; **132**: 569–585.

9 Arnhold J, Hammerschimdt S, Arnold K. Role of functional groups of human plasma and luminol in scavenging of NaOCl and neutrophil-derived hypochlorous acid. *Biochim Biophys Acta* 1991; **1097**: 145–151.

10 Onoyama K, Hirakata H, Iseki K, *et al.* Blood concentration and urinary excretion of captopril (SQ 14, 225) in patients with chronic renal failure. *Hypertension* 1981; **3**: 456–459.

11 Cody RJ, Schaer GL, Covit AB, Pondolfino K, Williams G. Captopril kinetics in chronic congestive heart failure. *Clin Pharmacol Ther* 1982; **32**: 721–726.

12 Clot P, Tabone M, Arico' S, Albano S. Monitoring oxidative damage in patients with liver cirrhosis and different daily alcohol intake. *Gut* 1994; **35**: 1637–1643.

13 Dillard CJ, Tappel AL. Fluorescent damage products of lipid peroxidation. *Methods Enzymol* 1984; **105**: 337–348.

14 Hunter MIS, Mohamed JB. Plasma antioxidants and lipid peroxidation products in Duchenne muscular distrophy. *Clin Chim Acta* 1986; **155**: 123–132.

15 Quinlan GJ, Evans TW, Gutteridge JMC. Linoleic acid and protein thiol changes suggestive of oxidative damage in the plasma of patients with adult respiratory distress syndrome. *Free Rad Res* 1994; **20**: 299–306.

16 Radi R, Bush KM, Cosgrove TP, Freeman BA. Reaction of xanthine oxidase-derived oxidants with lipid and protein of human plasma. *Arch Biochem Biophys* 1991; **286**: 117–125.

17 Kuzuya M, Yamada K, Hayashi T, *et al.* Oxidation of low density lipoprotein by copper and iron in phosphate buffer. *Biochim Biophys Acta* 1991; **1084:** 198–201.

18 Glantz SA. *Primer of Biostatistics*. New York: McGraw-Hill, 1987.

19 Kalyanaraman B, Sohnle PG. Generation of free radical intermediates from foreign compounds by neutrophil-derived oxidants. *J Clin Invest* 1985; **75**: 1618–1622.

20 Cannon PJ, Stason WB, Demartini FE, Sommers SC, Laragh JH. Hyperuricemia in primary and renal hypertension. *N Engl J Med* 1966; 275: 457–464.

21 Abrams J. Interactions between organic nitrates and thiol groups. *Am J Med* 1991; **91**: 106C-112C.

22 Egan TM, Minta JO, Scrimgeour KG, Cooper JD. Captopril-a potential free radical scavenger: inhibition of PMN NADPH oxidase. *Clin Invest Med* 1988; **11**: 351–356.

23 Sweet CS. Issues surrounding a local cardiac renin system and the beneficial actions of angiotensin-converting enzyme inhibitors in ischemic myocardium. *Am J Cardiol* 1990; **65**: 11I-13I.

24 Liao DF, Chen X. Prostacyclin-mediated protection by angiotensin-converting enzyme inhibitors against injury of aortic endothelium by free radicals. *Cardioscience* 1992; **3**: 79–84.

25 Wong KK, Lan S-J, Migdalof BH. In vitro biotransformations of [^{14}C]captopril in the blood of rats, dogs and humans. *Biochem Pharmacol* 1981; **19**: 2643–2650.
26 Godfrey EG, Stewart J, Dargie HJ, *et al.* Effects of ACE inhibitors on oxidation of human low density lipoprotein. *Br J Clin Pharmacol* 1994; **37**: 63–66.
27 Clearfield MB, Lee N, Armstrong L, DeFazio P, Kudchodkar BJ, Lacko AG. The effect of captopril on the oxidation of plasma lipoproteins. *Pharmacol Toxicol* 1994; **75**: 218–221.
28 Esterbauer H, Gebicki J, Puhl H, Jürgens G. The role of lipid peroxidation and antioxidants in oxidative modifications of LDL. *Free Radic Biol Med* 1992; **13**: 341–390.
29 Sobotka PA, Bottman MD, Weitz Z, Birnbaum AJ, Skosey JL, Zarling EJ. Elevated breath pentane in heart failure reduced by free radical scavenger. *Free Radic Biol Med* 1993; **14**: 643–647.
30 Cailleux A, Allain P. Is pentane a normal constituent of human breath? *Free Rad Res Commun* 1993; **18**: 323–327.
31 Springfield JR, Levitt MD. Pitfalls in the use of breath pentane measurements to assess lipid peroxidation. *J Lipid Res* 1994; **35**: 1497–1504.

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