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Original Contribution

BENEFICIAL EFFECTS OF α-LIPOIC ACID AND ASCORBIC ACID ON ENDOTHELIUM-DEPENDENT, NITRIC OXIDE-MEDIATED VASODILATION IN DIABETIC PATIENTS: RELATION TO PARAMETERS OF OXIDATIVE STRESS

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Abstract—The impairment of nitric oxide (NO)-mediated vasodilation in diabetes has been attributed to increased vascular oxidative stress. Lipoic acid has been shown to have substantial antioxidative properties. The aim of this study was to assess the effect of lipoic acid on NO-mediated vasodilation in diabetic patients in comparison with the well-recognized effect of ascorbic acid. Using venous occlusion plethysmography, we examined the effects of lipoic acid (0.2 mM) and ascorbic acid (1 and 10 mM) on forearm blood flow responses to acetylcholine, sodium nitroprusside and concomitant infusion of the NO-inhibitor, N^G-monomethyl-L-arginine, in 39 diabetic patients and 11 control subjects. Plasma levels of antioxidants and parameters of lipid peroxidation were measured and correlated to endothelial function tests. Lipoic acid improved NO-mediated vasodilation in diabetic patients, but not in controls. NO-mediated vasodilation was improved by ascorbic acid at 10 mM, but not 1 mM. Improvements of endothelial function by ascorbic acid and lipoic acid were closely related. The beneficial effects of lipoic acid were positively related to plasma levels of malondialdehyde and inversely related to levels of ubiquinol-10. These findings support the concept that oxidative stress contributes to endothelial dysfunction and suggest a therapeutic potential of lipoic acid particularly in patients with imbalance between increased oxidative stress and depleted antioxidant defense.

Keywords—α-Lipoic, Endothelium-derived nitric oxide, Diabetes mellitus, Oxidative stress, Free radicals

INTRODUCTION

The mechanism of endothelial dysfunction in diabetes mellitus is not known, but increased degradation of nitric oxide (NO) by oxygen-derived free radicals has been proposed [1–6]. In experimental models of diabetes, it has been shown that impaired endothelium-dependent vasodilation in both acute hyperglycemia [1] and chronic states of hyperglycemia [2,3] could be reversed by pretreatment with free radical scavengers and by administration of superoxide dismutase. In recent animal studies, excess production of superoxide anions was found within the vasculature [4,5] and reduction of superoxide production was associated with improvement of endothelial function. In patients with diabetes mellitus, endothelium-dependent vasodilation could be improved by a supraphysiological concentration of ascorbic acid [6].

Lipoic acid is a naturally occurring antioxidant with potent free-radical scavenging activity [7]. Incubation with lipoic acid has been shown to protect cultured endothelial cells against oxidative stress induced by high glucose [8] and to preserve cellular antioxidative defense mechanisms [9]. Furthermore, in diabetic animal models, lipoic acid has been demonstrated to have beneficial effects on vascular [10] and endothelial function [11]. However, the effect of lipoic acid on endothelium-dependent, NO-mediated vasodilation in humans has not been investigated.

The first aim of the present study was to investigate whether lipoic acid can improve endothelial function in diabetic patients. Second, we compared the effect of

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lipoic acid on endothelial function with the effect of the well-recognized antioxidant ascorbic acid. Third, plasma levels of antioxidants and parameters of lipid peroxidation were measured and correlated to endothelial function tests.

MATERIALS AND METHODS

Subjects

Eleven control subjects and 39 patients with Type II diabetes mellitus were studied. The known duration of diabetes was 4.2 ± 0.4 years (range from 2.5 to 7.3 years). Diabetic control was achieved by diet alone (n = 8) or diet plus oral hypoglycemic agents (n = 29) (metformin and/or sulfonylureas). Each subject was screened by a complete history, physical examination, and laboratory analysis. Exclusion criteria for both diabetic and control subjects included any of the following: hypertension (defined as blood pressure > 150/90 mmHg), hypercholesterolemia (defined as LDL-cholesterol > 75th percentile for age and sex), tobacco use within the past 5 years, current use of insulin, antioxidants or hormone replacement, and laboratory evidence of renal, hepatic, or hematological abnormalities. All female subjects were postmenopausal. The local ethics committee approved this study and informed consent was obtained from all participants.

Study protocol

All studies were performed after a 12-h overnight fast with the subjects lying supine in a quiet, temperature-controlled room (22–24°C). With the use of sterile conditions and 2% lidocaine, a 20-gauge polyethylene catheter was inserted into the nondominant brachial artery for measurement of blood pressure and infusion of drugs. Forearm blood flow (FBF) was measured by venous occlusion plethysmography with calibrated mercury-in-silastic strain gauges (D. E. Hokanson, Washington, DC, USA) connected to an analog-to-digital converter (McLab/4e, AD Instruments, Castle Hill, Australia) and a personal computer, as previously described [12]. Forearm volume was measured according to the water displacement method. At the beginning of each study protocol, normal saline (0.9% sodium chloride) was infused intra-arterially at a rate of 0.4 ml/min.

Protocol 1: Effect of lipoic acid on endothelium-dependent and endothelium-independent vasodilation

Endothelium-dependent vasodilation was assessed by infusing acetylcholine (ACh, Farmigea, Italy) in increasing concentrations of 0.75, 1.5, and 3.0 μg/100ml forearm tissue/min into the brachial artery. Sodium nitroprusside (SNP; Schwarz Pharma, Monheim, Germany) was infused to assess endothelium-independent vasodilation (0.1, 0.3, and 1.0 μg/100 ml forearm tissue/min). The sequence of ACh and SNP infusion was randomized. During coinfusion of NO-synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA 8 μmol/min, Calbiochem, Bad Soden, Germany), ACh response curve was repeated. This dose of L-NMMA has previously been shown to effectively blunt in vivo the synthesis of NO and thereby reduce ~40% of the vasodilator effect of acetylcholine in the human forearm [13–15]. The other component of ACh-induced increase of blood flow appears to be due to release of other vasodilators, such as prostacyclin and the endothelium-derived hyperpolarizing factor [16]. After a rest period of 30 min, lipoic acid (0.7 mg/min, ASTA Medica, Germany) was administered intra-arterially to all study participants to test the effects of lipoic acid on FBF response to ACh and SNP. Lipoic acid infusion was started 5 min before infusing ACh or SNP. Assuming a forearm blood flow of 30 ml/min, the local plasma concentration of lipoic acid was estimated to be of ~0.2 mM. This plasma level can be achieved by therapeutic intravenous infusion and has been shown to provide protective effects against oxidative stress-induced alterations in vitro [17,18]. Subsequently, dose-response curve to ACh was repeated during coinfusion of lipoic acid and L-NMMA. Glucose plasma levels were monitored during infusion of lipoic acid by drawing venous blood from the infused arm.

Protocol 2: Effect of ascorbic acid on endothelium-dependent and endothelium-independent vasodilation

A subgroup of 21 diabetic patients and 7 control subjects were retested on a separate day. Again, endothelium-dependent and endothelium-independent vasodilation was assessed by intra-arterial infusion of ACh and SNP. During coinfusion of L-NMMA (8 μmol/min), ACh response curve was repeated. After a rest period of 30 min, the ACh-induced vasodilation was tested again for each subject during coinfusion of ascorbic acid at 2.4 mg/min and 24 mg/min. The doses of ascorbic acid were chosen to provide calculated plasma concentrations of ~1 and 10 mM [19]. Ascorbic acid was started 10 min before ACh and continued throughout. Finally, the dose-response curve to ACh was repeated during coinfusion of ascorbic acid 24 mg/min and L-NMMA. A 30-min washout was allowed between each dose-response curve.

Plasma antioxidants and parameter of lipidperoxidation

Venous blood samples were drawn from each study participant before endothelial function tests. Samples for measurement of oxidation parameters were collected into EDTA sampling tubes and centrifuged, and plasma was stored at −80°C until used for analysis. Malondialdehyde (MDA) levels were determined using the method of Fukunaga [20]. This method is based on the thiobarbituric acid reaction and reversed-phase high performance liquid chromatography (HPLC) with fluorescence detection. The total radical-trapping antioxidant parameter (TRAP) measured was quantified by the method devised by Wayner et al. [21]. This method measures the overall capacity of human plasma to inhibit a radical-induced lipid peroxidation in vitro and is expressed as μmoles of lipid peroxyl radicals trapped by 1 l of plasma. Ubiquinol-10, ubiquinone-10, α- and γ-tocopherol were quantified in the same samples by HPLC with electrochemical detection as described elsewhere [22]. Sulfhydryl groups were analyzed photometrically.

Statistical analysis

All values are reported as mean ± SEM. Group comparisons with respect to baseline characteristics were performed by unpaired t test. Responses to ACh, SNP, and L-NMMA with and without lipoic acid or ascorbic acid were analyzed by ANOVA for repeated measures, and Scheffe's test was applied for multiple comparison testing. The relations between biochemical parameters, acetylcholine responses, and the effect of lipoic acid or ascorbic acid were examined by use of linear regression analysis. A value of $p < .05$ was considered statistically significant.

RESULTS

The clinical characteristics of the study groups are provided in Table 1. Diabetic patients and control subjects were matched for age and sex. Diabetic patients had significantly higher body mass index, fasting glucose, glycated haemoglobin, mean blood pressure, and triglyceride levels. The serum cholesterol concentrations were similar in both groups. Forearm volume and basal forearm blood flow were not different between diabetic and control subjects.

Table 1. Clinical Characteristics of Study Groups

	Diabetic patients	Control subjects
n	39	11
Sex (m/f)	28/11	8/3
Age (years)	56 ± 4	52 ± 3
Duration of diabetes (years)	4.2 ± 0.6	–
BMI (kg/m^2)	26.3 ± 0.4*	24.5 ± 0.5
Fasting glucose (mmol/l)	9.0 ± 0.6*	4.8 ± 0.2
HbA1c (%)	7.5 ± 0.2*	5.2 ± 0.2
Total cholesterol (mmol/l)	5.4 ± 0.2	5.5 ± 0.3
LDL-cholesterol (mmol/l)	3.4 ± 0.2	3.6 ± 0.3
HDL-cholesterol (mmol/l)	1.2 ± 0.08	1.1 ± 0.10
Triglycerides (mmol/l)	1.9 ± 0.3*	1.5 ± 0.4
Mean blood pressure (mmHg)	93 ± 3*	86 ± 4
Forearm volume (l)	1.06 ± 0.03	1.03 ± 0.05
Forearm blood flow (ml/100 ml/min)	2.9 ± 0.1	3.1 ± 0.1

Data presented as means ± SEM. *$p < .05$ vs. control subjects.

Effect of lipoic acid on endothelium-dependent and endothelium-independent vasodilation

As shown in Fig. 1, acetylcholine (ACh) dose-dependently increased forearm blood flow (FBF) in both groups. However, the vasodilator response in diabetic patients (from 2.9 ± 0.1 to maximal 10.2 ± 1.2 ml/100 ml/min) was significantly lower compared to normal subjects (from 3.1 ± 0.1 to maximal 17.6 ± 1.3 ml/100 m/min) ($p < .01$ by ANOVA). Co-infusion of the NOS-inhibitor L-NMMA blunted the vasodilating effect of ACh significantly in control subjects. In diabetic patients, however, L-NMMA had no significant effect. Infusion of lipoic acid did not change basal FBF in either group. In control subjects, co-infusion of lipoic acid had no effect on ACh-induced vasodilation. However, in diabetic patients, ACh-induced vasodilation was significantly enhanced by concomitant administration of lipoic acid (maximal FBF before lipoic acid: 10.2 ± 1.2 vs. after lipoic acid: 14.2 ± 1.2 ml/100 ml/min). When the effect of L-NMMA was retested in the presence of lipoic acid, the NOS-inhibitor completely abolished the vasodilatory effect of lipoic acid in diabetic patients (maximal FBF: 9.8 ± 1.3 ml/100 ml/min). In control subjects, lipoic acid did not alter the inhibitory effect of L-NMMA on the ACh dose-response curve. The FBF response to SNP was similar in control subjects (maximal FBF: 14.3 ± 1.3 ml/100 ml/min) and diabetic patients (maximal FBF: 13.4 ± 1.3 ml/100 ml/min) and was not modified by lipoic acid (Fig. 2). Monitored glucose plasma levels did not change during infusion of lipoic acid.

Effect of ascorbic acid on endothelium-dependent and endothelium-independent vasodilation

As shown in Fig. 3 (left panel), ascorbic acid infusion at 2.4 mg/min had no effect on FBF response to ACh in diabetic patients. In contrast, ascorbic acid infusion at 24 mg/min improved FBF response to ACh in diabetic patients ($p < .01$) (Fig. 3, right panel). The response to the highest

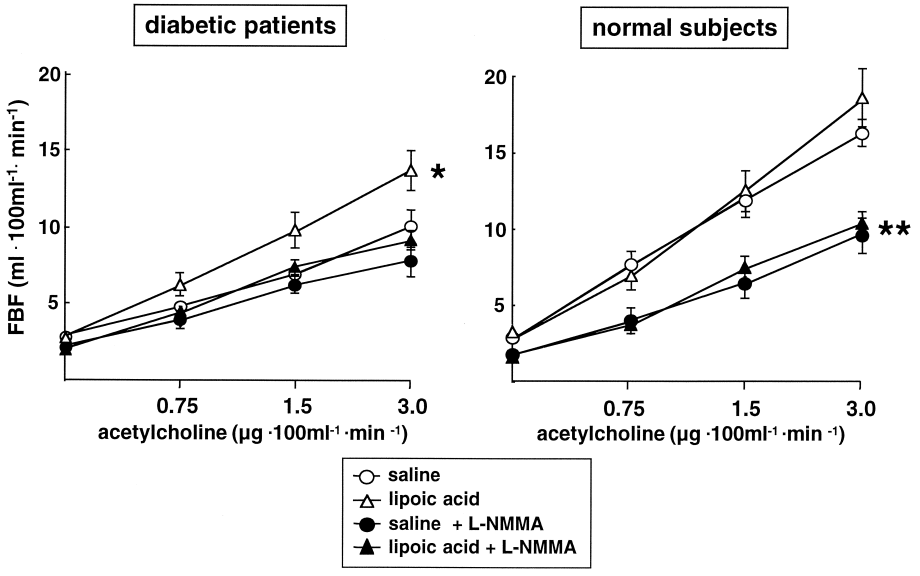

Fig. 1. Effect of lipoic acid on acetylcholine-induced vasodilation in diabetic patients and control subjects with and without coinfusion of L-NMMA. Lipoic acid significantly improved acetylcholine-induced vasodilation in diabetic patients, but did not change the dose-response curve in control subjects. In diabetic patients, coinfusion of L-NMMA had no effect under control conditions (saline), but completely blocked the lipoic acid-induced increase in forearm blood flow. In control subjects, the effect of L-NMMA on the dose-response curve was not altered by lipoic acid. Data are shown as means ± SEM. *Significant difference in the overall dose response vs. saline and L-NMMA-coinfusion ($p < .05$). **Significant difference between infusions with and without L-NMMA ($p < .05$).

dose of ACh was 10.8 ± 1.4 ml/min/100 ml before and 14.1 ± 1.4 ml/min/100 ml during coinfusion of ascorbic acid. Coinfusion of the NOS-inhibitor L-NMMA had no effect before ascorbic acid, but completely blocked the vasodilatory effect of ascorbic acid 24 mg/min. In control subjects, however, neither dose of ascorbic acid had any significant effect on endothelium-dependent vasodilation (data not shown).

In diabetic patients, the individual improvement of ACh-induced FBF by ascorbic acid and lipoic acid varied considerably. There was a close relationship between improvement of endothelial function by ascorbic acid and the beneficial effect of lipoic acid ($r = 0.83$, $p < .001$) (Fig. 4).

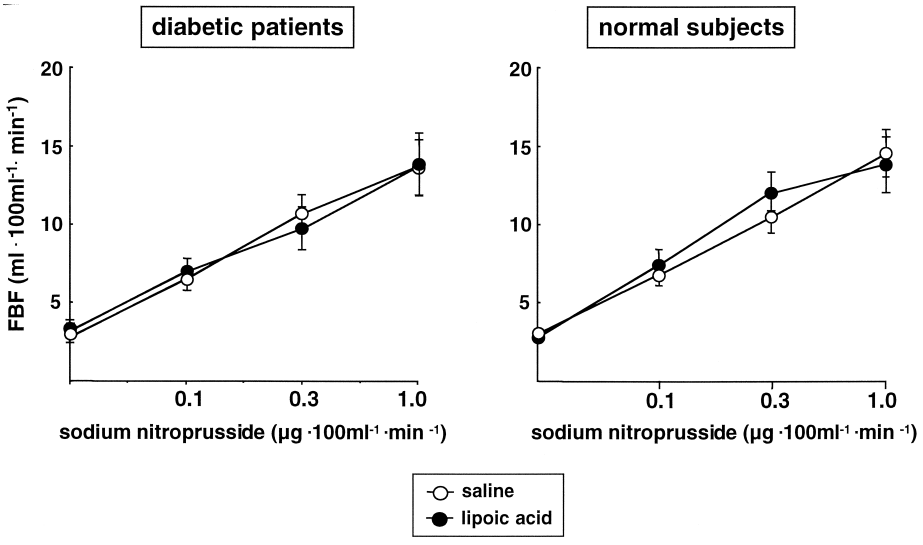

Fig. 2. Effect of lipoic acid on sodium nitroprusside-induced vasodilation in diabetic patients and control subjects. Lipoic acid did not modify sodium nitroprusside-induced vasodilation in either diabetic patients or control subjects. Data are shown as means ± SEM.

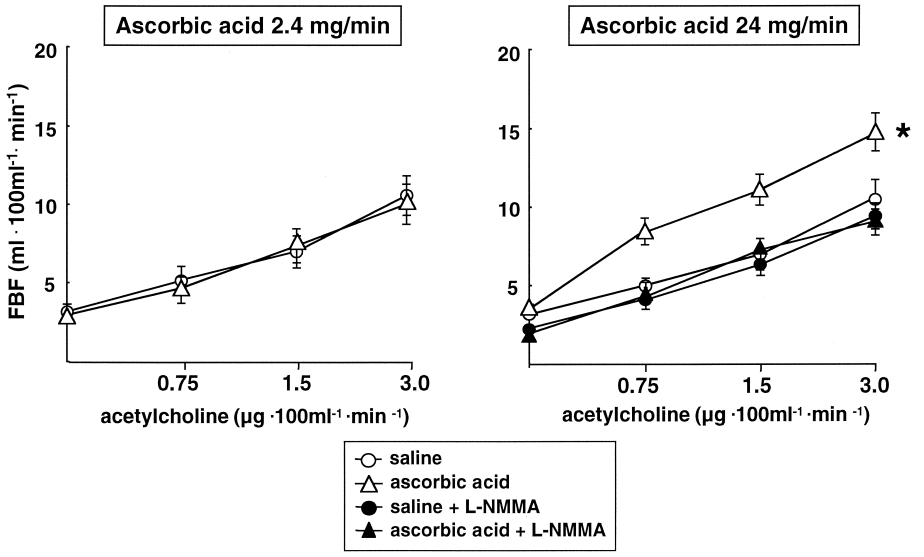

Fig. 3. Effect of ascorbic acid at 2.4 mg/min and 24 mg/min on acetylcholine-induced vasodilation in diabetic patients. Ascorbic acid at 2.4 mg/min had no effect on acetylcholine-induced vasodilation (p = ns), whereas ascorbic acid at 24 mg/min significantly increased the forearm blood flow response to acetylcholine. Confusion of L-NMMA had no effect under control conditions (saline), but completely blocked the ascorbic acid-induced increase in forearm blood flow. Data are shown as means ± SEM. *Significant difference in the overall dose response vs. saline and L-NMMA-coinfusion ($p < .01$).

Plasma oxidation parameter and correlation to endothelial function

Plasma levels of TRAP, α- and γ-tocopherol were comparable between both study groups (Table 2). Plasma concentration of malondialdehyde (MDA) was significantly higher in diabetic patients compared to control subjects ($p < .05$). The plasma level of SH groups was lower in diabetic patients ($p < .01$). Ubiquinol-10, the reduced form of coenzyme Q10, was lower in diabetic patients compared to controls, both when expressed as a percentage of total coenzyme Q10 ($p < .05$) and when normalized to total plasma cholesterol and triglycerides ($p < .01$). There was no relationship between endothelium-dependent vasodilation and plasma levels of MDA, TRAP, SH-groups, α- and γ-tocopherol, or ubiquinol-10 in diabetic patients and control subjects. However, the effect of lipoic acid on endothelium-dependent vasodilation in diabetic patients was significantly correlated to plasma levels of MDA ($r = 0.61$, $p < .001$) (Fig. 5) and plasma concentrations of ubiquinol-10, normalized to total cholesterol and triglycerides ($r = 0.57$, $p < .01$).

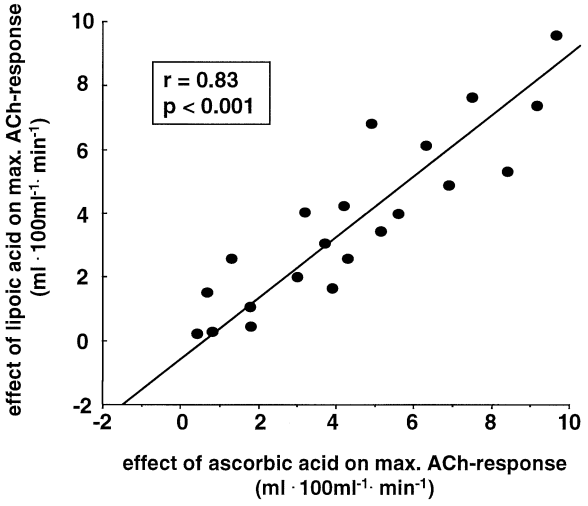

Fig. 4. Correlation between the effect of lipoic acid (0.7 mg/min) and ascorbic acid (24 mg/min) in a subgroup of 21 diabetic patients. The effect of lipoic acid and ascorbic acid are given as the difference between the maximal acetylcholine-induced forearm blood flow response during lipoic acid or ascorbic acid and corresponding saline infusion.

Table 2. Biochemical Data of Study Groups

	Diabetic patients (n = 39)	Control subjects (n = 11)
TRAP (µmol/l)	846 ± 34	807 ± 57
MDA (µmol/l)	0.74 ± 0.05*	0.57 ± 0.3
SH-groups (µmol/l)	368 ± 13*	465 ± 24
Ubiquinol-10/coenzyme-Q10 (%)	72.4 ± 6.9*	84.3 ± 8.4
Ubiquinol-10/chol+tg (nmol/mmol)	76 ± 7*	108 ± 8
α-tocopherol (µmol/l)	24.7 ± 1.1	23.2 ± 1.2
α-tocopherol/chol+tg (µmol/mmol)	4.2 ± 0.3	4.0 ± 0.4
γ-tocopherol (µmol/l)	2.0 ± 0.1	1.8 ± 0.2
γ-tocopherol/chol+tg (µmol/mmol)	0.31 ± 0.02	0.30 ± 0.03

Data presented as means ± SEM. *$p < .05$ vs. control subjects.
TRAP = total radical-trapping antioxidant parameter, MDA = malondialdehyde, SH = sulfhydryl groups, chol+tg = total cholesterol + triglyceride.

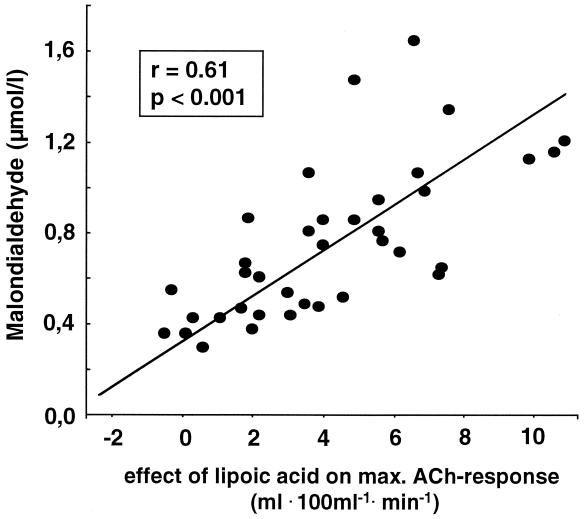

Fig. 5. Correlation between plasma levels of malondialdehyde and the effect of lipoic acid (0.7mg/min) in diabetic patients (n = 37). The effect of lipoic acid is given as the difference between the maximal acetylcholine-induced forearm blood flow during lipoic acid and saline.

Patients with higher MDA levels or lower ubiquinol-10 plasma concentrations were more likely to have improved endothelium-dependent vasodilation by administration of lipoic acid.

DISCUSSION

This study demonstrates that lipoic acid (LA) in a therapeutic dose improves endothelial dysfunction in diabetic patients by increasing nitric oxide (NO)-mediated vasodilation. In contrast, a supraphysiological concentration of ascorbic acid is required to achieve a comparable improvement. The effect of LA was significantly related to plasma levels of malondialdehyde and ubiquinol; thus patients with higher levels of malondialdehyde and lower concentrations of ubiquinol were more likely to benefit from LA.

The findings of the present study support the results of previous investigations demonstrating impaired forearm blood flow responses to endothelium-dependent vasodilators in patients with Type II diabetes mellitus [6,14,15]. In addition, although intra-arterial administration of the NOS inhibitor L-NMMA clearly inhibited the vasodilating effect of ACh in control subjects, it was found to be ineffective in diabetic patients. This finding, together with the normal response of vascular smooth muscle to the endothelium-independent vasodilator SNP, implicates reduced endothelium-derived NO bioavailability and is in line with previous reports of endothelial dysfunction in diabetic patients [14,15].

Although endothelial dysfunction in diabetes mellitus is very likely multifactorial, more recent clinical and experimental observations point to a potential role of increased vascular oxidative stress as an important component of this phenomenon [1–6]. In animal models of diabetes, endothelial dysfunction was shown to be associated with increased oxygen-derived free radical production and could be restored by treatment with antioxidants [1–5]. These experimental findings support the notion that reactive oxygen species may inactivate endothelial NO and lead to endothelial dysfunction in the diabetic state.

There is also accumulating evidence for increased oxidative stress in patients with diabetes. The susceptibility of LDL to oxidative modification has been reported to be increased in diabetic patients [23,24]. The plasma from diabetic subjects contains increased levels of malondialdehyde [25,26], lipid hydroperoxides [27], and F_2-isoprostanes [28], markers of lipid peroxidation. In addition, a number of studies have found depletion of antioxidants such as vitamin E [27], thiol groups [26], and ubiquinol-10 [29]. In the present study, diabetic patients had higher plasma levels of malondialdehyde and lower serum concentrations of SH-groups and ubiquinol-10 compared to controls. These findings are similar to results of other investigators [26,29] and consistent with the concept of increased oxidative stress in diabetes.

Lipoic acid, which plays an essential role in mitochondrial dehydrogenase reactions, has recently gained considerable attention as an antioxidant. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), reacts with reactive oxygen species such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen [7]. Lipoic acid and DHLA have also been shown in vitro to be capable of regenerating other antioxidants such as ascorbic acid, glutathione, ubiquinol-10, and, indirectly, vitamin E [30]. Several experimental and animal studies have been conducted to prove its antioxidant potency [7]. However, there are only few data available on the antioxidant effect of LA in humans. Lipoic acid is soluble in both lipid and aqueous environments, is readily absorbed from the diet, taken up by cells, and rapidly reduced to DHLA in many tissues [7]. Recently, it has been demonstrated in diabetic patients, that oral supplementation of LA decreases oxidative stress, assessed by measurement of plasma lipid hydroperoxides [31]. Another study found that oral supplementation of LA reduces urinary F2-isoprostanes as well as LDL oxidizability [32].

The present study is the first report that administration of LA in a therapeutic dose improves endothelium-dependent vasodilation in diabetic patients. A nonspecific improvement of the vasodilatory capacity of forearm resistance vessels is not very likely, since LA had no significant effects on endothelium-independent vasodila-

tion induced by sodium nitroprusside. Furthermore, the beneficial effects of LA could be blocked by NOS-inhibitor L-NMMA, indicating that in the presence of LA, the activity of the L-arginine-NO pathway is increased.

The potential mechanisms by which LA may modulate endogenous NO bioactivity are not yet clear. Due to its antioxidant potency, LA, particularly in its reduced form of DHLA, may spare NO by directly scavenging oxygen-derived free radicals. We therefore compared the effect of LA with the effect of the well-recognized antioxidant ascorbic acid. The finding of a strong relationship between the effect of both substances points to the suggestion that both agents may act by a similar mechanism. However, somewhat surprising is the large difference in the required concentration of LA (0.2 mM) and ascorbic acid (10 mM) to achieve comparable improvement of NO bioactivity. Recent studies on endothelial function confirm our finding that high pharmacological concentrations of vitamin C have to be infused to improve NO bioactivity, whereas lower concentrations fail to achieve an improvement [19]. Similarly, using an in vitro model of superoxide-mediated vascular dysfunction, it was shown that an ascorbic acid concentration of 10 mM was required to restore endothelium-dependent vasodilation, whereas a concentration of 1 mM was ineffective under these conditions [33]. The most likely explanation for this finding is given by the large difference in rate constants: the rate constant for the reaction between superoxide radicals and ascorbic acid ($2.7–3.3 \times 10^5$ M^{-1} s^{-1}) is approximately 10^5-fold less than the extremely rapid reaction rate between NO and superoxide anion (1.9×10^{10} M^{-1} s^{-1}). Therefore, only high concentrations of ascorbic acid ($>$ 1 mmol/l) compete effectively with NO for superoxide anions. In the present study, however, LA improved NO activity at the much lower concentration of 0.2 mM in spite of a very similar reaction constant with superoxide radicals of 1.4×10^5 M^{-1} s^{-1} compared to ascorbic acid [34]. This difference between LA and ascorbic acid may be due to better uptake of LA into endothelial cells or due to additional mechanisms of LA compared to ascorbic acid. With respect to the former mechanism, ascorbic acid is actively transported into cells and accumulates intracellularly up to concentrations of 1.3–2.5 mmol/l [35]. However, as ascorbic acid is a very hydrophilic agent, a substantial intracellular transport of ascorbic acid is unlikely to occur during the short time course of an acute experiment [33]. In contrast, LA has both hydrophilic and lipophilic properties and is readily taken up by cells and converted to DHLA in many tissues. It was found that intracellular concentrations of DHLA reached 1.5–2 mM within 5–10 min in experimental cell studies [36]. Taken together, although the exact mechanism of uptake and metabolism of lipoic acid in humans is not known, the beneficial effects of LA 0.2 mM could be due to a higher intracellular availability of LA in comparison to ascorbic acid.

In addition to its direct scavenging activity, LA may modulate endogenous NO bioactivity by its known ability to increase intracellular levels of glutathione [37]. Although the precise mechanisms are not yet clear, increasing glutathione availability has been shown to enhance NO synthesis within endothelial cells [38] and to improve endothelium-dependent vasodilation in humans [39]. Glutathione plays a central role in regulation of the intracellular redox state [40]. In this regard, there is a growing body of evidence that intracellular redox state has substantial influence on endothelial NO-synthase activity by modulating tetrahydrobiopterin availability. Tetrahydrobiopterin, an essential cofactor for endothelial NOS, is readily oxidized, and tetrahydrobiopterin deficiency has been demonstrated to cause an uncoupling of the endothelial NOS, thereby generating superoxide instead of NO [41]. Indeed, supplementation of tetrahydrobiopterin has been shown to block superoxide production and to restore abnormal endothelium-dependent vasodilation in aortas of diabetic rats [5] and to improve endothelium-dependent vasodilation in patients with diabetes [15]. Therefore, by improving intracellular redox state, both ascorbic acid and LA may protect tetrahydrobiopterin from oxidation, thereby enhancing endothelial cell capacity for NO production. Most interestingly, recent experimental studies demonstrated that intracellular ascorbic acid potentiates endothelial NOS activity by increasing intracellular tetrahydrobiopterin availability [42].

In our study, although plasma oxidation parameters were altered in diabetic patients, we found no correlation between oxidant stress markers and impairment of endothelial function. However, plasma oxidation parameter such as malondialdehyde and ubiquinol-10 were significantly related to the LA-induced improvement of endothelial function. This is consistent with the notion that enhancement in NO-mediated vasodilation, most likely via scavenging reactive free radicals, can lead to a recovery in diabetic endothelial function and might be particularly beneficial in a subgroup of diabetic patients, that is, individuals with elevated levels of lipid peroxidation and/or decreased antioxidant defense.

In conclusion, short-term treatment with LA in a therapeutic dose improved endothelium-dependent and nitric oxide-mediated vasodilation in diabetic patients. Ascorbic acid achieved a similar improvement at a high supraphysiological concentration. These findings support the notion that oxygen free radicals contribute to endothelial dysfunction in diabetes and provide a rationale for

the therapeutic potential of LA in endothelial dysfunction and impaired NO bioactivity.

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