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REGULAR ARTICLE

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Beneficial effects of L-arginine supplementation in experimental hyperlipemia-hyperglycemia in the hamster

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Abstract The objective of this study was to evaluate whether administration of L-arginine, the substrate for nitric oxide synthesis, was able to ameliorate the endothelial dysfunction and the morphological changes induced by the combined insult of hyperlipemia and hyperglycemia. To this purpose, golden Syrian hamsters were rendered simultaneously hyperlipemic and diabetic (HD group) for 24 weeks, and then orally treated with 622.14 mg/kg per day L-arginine, for 12 weeks (HD + L-arg group). The following assays were carried out: (1) spectrophotometric: concentrations of circulating glucose, cholesterol, and creatinine, the activity of angiotensin-converting enzyme (ACE), and the osmotic fragility of erythrocyte plasmalemma; (2) myographic: the endothelium-dependent and -independent relaxation of the resistance arteries (i.d. 210–250 μm) to 10^{-8} to 10^{-4} M acetylcholine (ACh) or sodium nitroprusside (SNP); and (3) electron-microscopic: the ultrastructure of the resistance arteries, myocardium, and kidney glomeruli, which are main targets of hypertensive complications.The results showed that oral supplementation with L-arginine in simultaneous hyperlipemia-hyperglycemia induced in hamsters had favorable effects on: (1) homeostasis, i.e., diminished the concentration of circulating glucose (by ~63%) and cholesterol (by ~10%), reduced the ACE activity (by ~45%), and lowered the osmotic fragility of erythrocyte plasmalemma (as marker for the oxidative stress in plasma); (2) mesenteric resistance arteries, which showed (in 10^{-4} M ACh) an improved endothelium-dependent relaxation (72.40±4.6% in the HD + L-arg group vs 61.90±1.45% in the HD group) and a reduced thickness (~1.32-fold) of the smooth muscle cells' extracellular matrix; and (3) the heart, which displayed ~16% diminishment of the thickness of the left ventricular wall, and an apparently normal structure of the myocardium; the restoration of the thickness of the pericapillary extracellular matrix to almost normal dimensions was also observed. Administration of L-arginine did not modify the high level of plasma creatinine determined for the HD group (~48% increased vs control group) and had no effect on the thickened, nodular basal lamina of the kidney capillaries. The results indicate that endothelial dysfunction established in combined hyperlipemia-diabetes is distinctive for each vascular bed (mesenteric arterioles, heart capillaries, kidney glomerular capillaries), and there is a reversible stage of the dysfunction in which L-arginine oral supplementation induced beneficial effects.

Keywords Simultaneous hyperlipemia-diabetes · Resistance arteries · Vascular tonus · Heart capillaries · Glomerular capillaries · Syrian hamster

Introduction

In response to either physical factors (shear stress, pulsatile stretching of the vessel wall) or vasoactive substances (circulating or locally released), the endothelial cell layer maintains normal vascular homeostasis by a balanced production of endothelium-derived relaxing and contracting factors (Herrmann and Lerman 2001), of anti- and procoagulant mediators, and of growth-inhibiting and growth-promoting factors (Rubanyi 1993). This balance is disturbed in pathological conditions such as atherosclerosis, diabetes, and hypertension, and the "endothelial dysfunction" becomes manifest often as an early preclinical event (Rakugi et al. 1996; Cines et al. 1998; Pieper et al. 1998; Shimokawa 1998, 1999; Simon et al. 1999; Thalhammer et al. 1999; Herrmann and Lerman 2001). A common feature of endothelial dysfunction is the reduced vasodilation in response to pharmacological endothelium-dependent stimuli, a process

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that involves the endogenous messenger molecule nitric oxide (NO). Based on altered biochemical mechanisms unraveled so far, new strategies designed to "treat" endothelial dysfunction (Stehouwer 1999) are now in progress. Interestingly, recent findings show that administration of L-arginine (the natural precursor of vascular NO) ameliorates endothelium-dependent vasodilation not only in animal models of hypercholesterolemia and atherosclerosis, but also in patients, along with an improvement of the clinical symptoms of human cardiovascular disease (Böger and Bode-Böger 2001). The large survey published by Cheng and Balwin (2001) points out the favorable role of L-arginine in the management of multiple cardiovascular diseases and suggests that more experimental studies are still necessary before its recommendation in human therapy.

In this report we investigated the effects of L-arginine (administrated orally) in simultaneous hyperlipemia and hyperglycemia (induced in hamsters), an experimental model that mimics diabetes complicated with accelerated atherosclerosis (Simionescu et al. 1996; Costache et al. 2000). We hypothesized that L-arginine would increase the production of NO in microvascular and arteriolar endothelium (heart and kidney capillaries, and mesenteric resistance arteries), and thus ameliorate the pathological changes determined by the simultaneous insult of hyperlipemia-diabetes.

Materials and methods

Experimental animals

Fifty male golden Syrian hamsters, 105±12 g body weight, were divided into two experimental groups: (1) simultaneously hyperlipemic and diabetic (HD group), fed a standard chow to which 3% cholesterol and 15% butter were added, and subjected to one i.p. injection of streptozotocin (50 mg/kg, dissolved in 50 mM citrate buffer, pH 4.5); and (2) controls (C group), consisting of age-matched animals that received an i.p. injection of the vehicle solution only. At 12 weeks after the initiation of the experiment, hamsters in the HD group received by gavage a daily dose of 3 mM L-arginine in 125 µl saline solution (HD + L-arg group), corresponding to 622.14 mg L-arginine/kg. No insulin was given to groups HD ± L-arg to correct hyperglycemia. The animal experiments were performed in accordance with *Principles of laboratory animal care* (NIH publication no. 83–25, revised 1985).

Ratios heart weight to body weight, and kidney weight to body weight

For each animal in groups C and HD ± L-arg, the heart and left kidney were removed, blotted dry, and weighed, and the ratios heart weight to body weight (as index of left ventricular hypertrophy; Shaw et al. 1995) and kidney weight to body weight were calculated.

Biochemical assays

Hamsters were slightly anesthetized by an i.p. injection of 5% chloralhydrate (0.05 ml/100 g), which induced analgesic and weak hypnotic effects. Then, blood was collected sterilely from the venous retro-orbital plexus. Plasma (blood collected on 2.7 mM EDTA) was used for the measurement of glucose, cholesterol, and creatinine levels (using the Sigma kits). The activity of angiotensin-converting enzyme (ACE) was evaluated in serum as described by Schnaith et al. (1994).

To assay the osmotic fragility of erythrocytes, the procedure described by Kowluru et al. (1996) was used. Briefly, blood collected on heparin was brought to 5% hematocrit, and the erythrocytes were incubated for 30 min at 37°C with 200 mosmol/l NaCl. The latter osmolarity was selected from preliminary experiments using erythrocytes exposed to 80–320 mosmol/l NaCl. After centrifugation, the hemoglobin released was measured as O.D. at 412 nm, and the values determined from normal animals were taken as 100%.

Vascular reactivity of the resistance arteries

Animals were killed by cervical dislocation, and after laparotomy the small intestine (7–10 cm from the pylorus) was excised. Segments (2–3 mm long) of the second- and third-order branches of the mesenteric arteries were dissected out, threaded through two stainless steel wires (i.d. 40 µm), and mounted in a small wire myograph (model 410 A; JP Trading, Denmark) as described by Mulvany and Halpern (1977). The mean i.d. of the arteries used was in the range of 210–250 µm (resistance arteries). The myograph chamber was filled with HEPES salt solution (as described by Chulia et al. 1995 and Thurston et al. 1995), maintained at 37°C and continuously gassed with O_2. After an equilibration period of 20 min, the arteries were set to the normalized internal circumference (0.9 times the circumference they would have when relaxed and subjected to 100 mmHg). A routine "run-up" procedure consisting of consecutive contractions to noradrenaline (NA; 5 µmol NA/l), 125 mmol K^+/l, and 5 µmol NA/l in 125 mmol K^+/l was performed for each vessel (Palmer et al. 1998). To measure the endothelium-dependent and -independent relaxation of the resistance arteries, vessels precontracted in 10^{-4}M NA (at ~80% of the maximal force) were exposed (every 2 min) to cumulative concentrations (10^{-8} to 10^{-4}M) of either acetylcholine (ACh) or sodium nitroprusside (SNP). The vasodilation was either monitored on a chart-recorder or quantitated in milliNewtons (mN) at the interface of the myograph. In some experiments the activity of nitric oxide synthase (NOS) was inhibited with *N*-ω-nitro-L-arginine methylester (L-NAME, 10^{-4} M) added to the organ bath for 45 min prior to ACh concentration-response measurements.

Tissue preparation for electron microscopy

Hamsters in either C or HD ± L-arg groups were anesthetized by an i.p. injection of 5% chloral hydrate (0.1 ml/100 g body weight). After laparotomy and catheterization of the abdominal aorta, the vena cava caudalis was punctured, and the blood was washed out by perfusion of phosphate-buffered saline at a flow rate of 6 ml/min. Under the same conditions, the tissues were fixed by perfusing a mixture of 2.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer, supplemented with 2.5 mmol/l $CaCl_2$, pH 7.4. After 10 min, small fragments of the second- and third-order branches of mesenteric arteries, of heart left ventricle, and of kidney cortex were removed and processed separately. After 90 min in fixative, the tissue fragments were postfixed in 1% OsO_4, dehydrated in graded concentrations of ethanol, and embedded in Epon 812. Thin sections stained with 7.6% uranyl acetate (in distilled water) and 0.4% lead citrate (in 0.1 N NaOH) were examined with the Phillips electron microscope (Eindhoven, The Netherlands).

Morphometric analysis

To quantitate the thickness of the endothelial basal lamina of myocardial and kidney capillaries, the method of Williamson et al. (1976) was employed on pictures at similar magnification (×55,000).

Table 1 Effect of administration of L-arginine (622.14 mg/kg per day for 12 weeks) on simultaneous hyperlipemic-diabetic hamsters (HD, 24 weeks into the experiment), as compared to age-matched controls. Each value represents the mean±SEM of 5–11 animals

Experimental condition	HD group	HD + L-arg group	C group
Plasma glucose (mg/dl)	265±31[1]*	96.14±22.79[2*,3*]	94.72±16.98
Plasma cholesterol (mg/dl)	748±80[1]*	675±31.00[1*,4*]	75.30±4.42
Plasma creatinine (μM)	127.15±2.25[1]*	114.53±5.83[1*,4*]	65.91±4.21
Serum ACE activity (μmol/ml·min)	51.34±2.10[1]*	28.33±5.31[2*]	20.25±7.36
Erythrocytes: osmotic fragility (% of controls)	488.70±34.2[1*]	303.33±84[1*,2*]	100.00
Heart weight/body weight × 10^{-2}	0.511±0.01[1*]	0.472±0.024[4*]	0.449±0.014
Resistance arteries: relaxation in 10^{-4} M ACh (% from NA precontraction)	61.94±1.45[1*]	72.40±4.60[2*,3*]	80.20±3.20
Kidney weight/body weight × 10^{-2}	0.745±0.05[1*]	0.729±0.14[1*,4*]	0.530±0.02

[1]**P*<0.05 compared with the C group; [2]**P*<0.05 compared with the HD group; [3]*non-significant difference versus C group; [4]*non-significant difference versus HD group

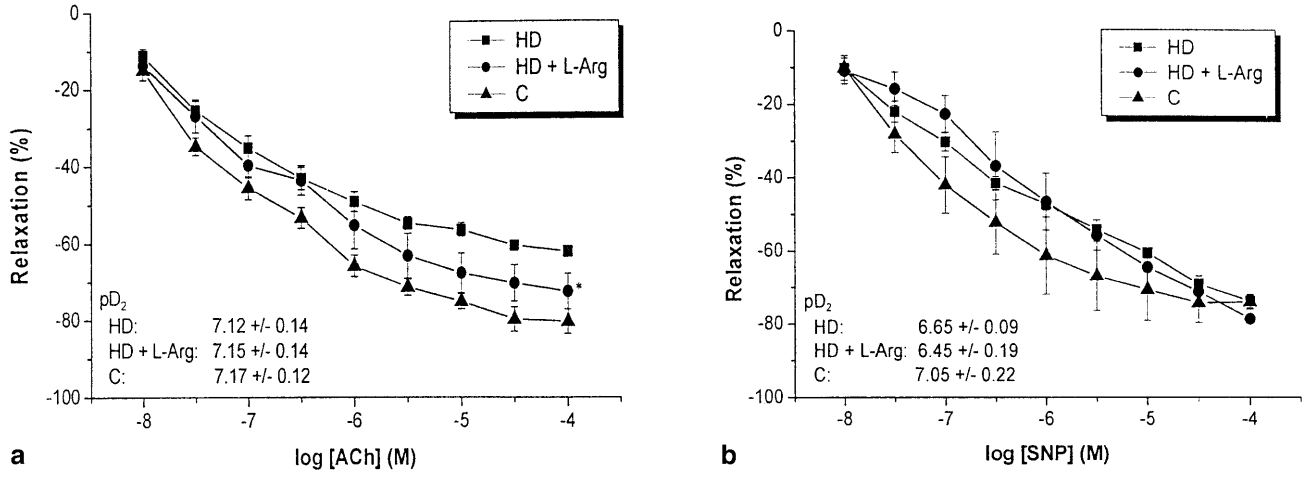


Fig. 1a, b Endothelium-dependent relaxation of the resistance arteries to increasing doses of 10^{-8} to 10^{-4} M acetylcholine (*ACh* in **a**); endothelium-independent relaxation to cumulative concentrations of 10^{-8} to 10^{-4} M sodium nitroprusside (*SNP* in **b**). The numbers of animals used were: 8 for control group, 11 for hyperlipemic and diabetic (HD) group, and 5 for HD + L-arginine group. For each animal at least two arteries were tested in the dual-chamber of the myograph (pD_2 negative logarithm of the molar concentration required to produce 50% of maximal response)

Statistical analysis

The results on the vascular reactivity were expressed as the mean value ± SEM. The contractile tension of the resistance arteries to NA was expressed as active wall tension (milliNewtons per millimeter artery length) ± SEM. The relaxation responses to ACh and SNP were calculated as percentage of the force developed at the initial precontraction in NA. The sensitivity of the arteries' response to ACh was expressed as pD_2, i.e., the negative logarithm of the molar concentration required to produce 50% of the maximal response. All the concentration-response curves were compared by one-way ANOVA. Significance was considered if $P<0.05$.

Results

Effect of L-arginine on the general homeostasis of hyperlipemic-diabetic hamsters

In the HD + L-arg group, the circulating glucose concentration was 96.14±22.79 mg/dl, i.e., ~63% diminished versus HD group; correspondingly, the ACE activity was found to be reduced by ~45% (Table 1). A slight decrease in cholesterol level (~10%) was determined in the HD + L-arg group (vs HD group), while the plasma creatinine remained still high after L-arginine administration (~43% in the HD + L-arg group and ~48% in the HD group versus C group; Table 1).

Taking the erythrocytes' lysis in the C group as 100% (Kowluru et al. 1996), the osmotic fragility of erythrocytes in the HD group was ~4.88-fold enhanced, while in the HD + L-arg group a ~3-fold increase only was recorded (Table 1).

Effect of L-arginine on the mesenteric resistance arteries

The relaxation of the vascular wall

The mesenteric resistance arteries precontracted in NA relaxed to the endothelium-dependent vasodilator ACh (10^{-8} to 10^{-4} M) to a maximum of 80.20±3.20% in the C group, to 61.94±1.45% in the HD group ($P<0.001$ vs C group), and to 72.40±4.60% in the HD + L-arg group ($P=0.026$ vs HD group; Fig. 1a). There was no change in

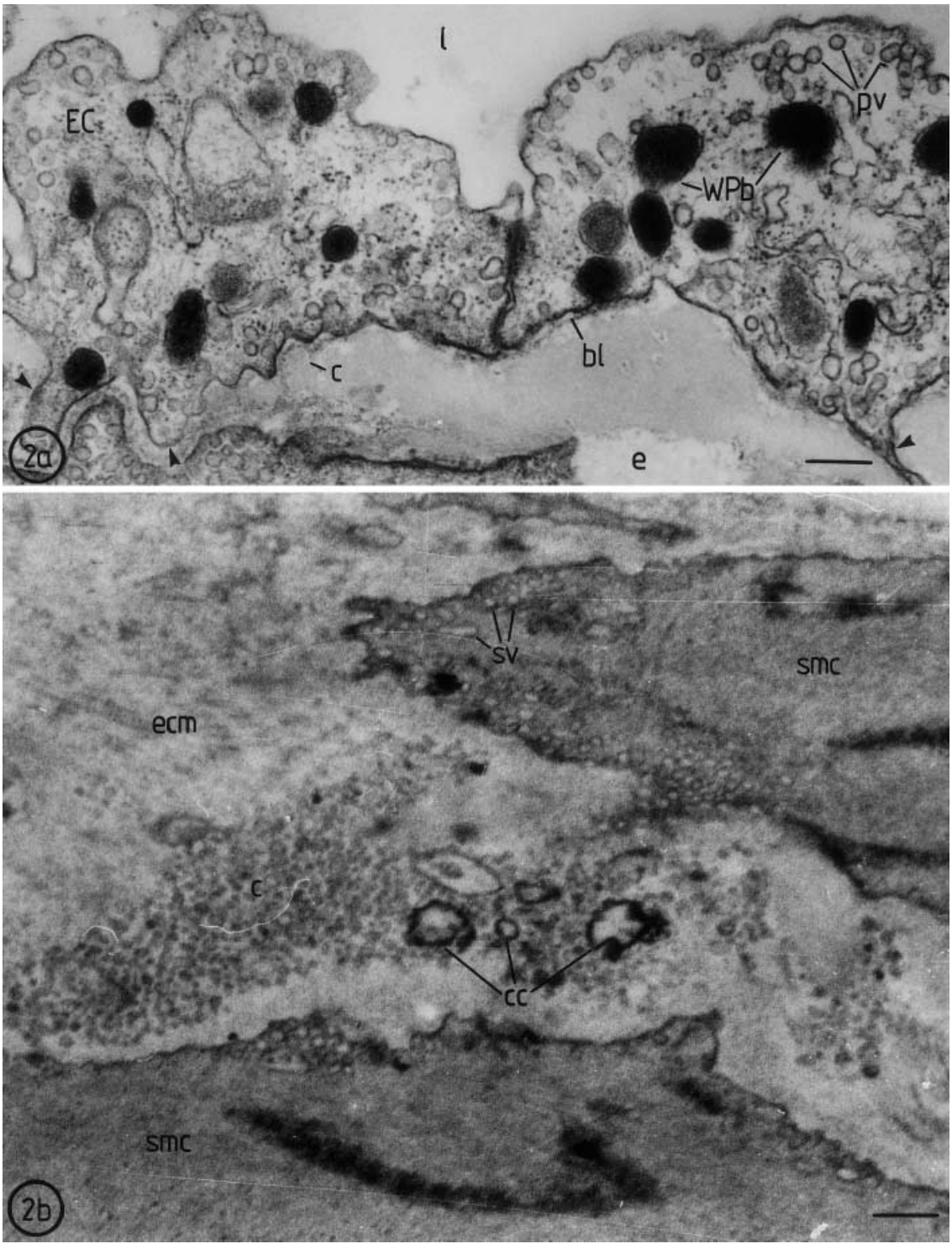
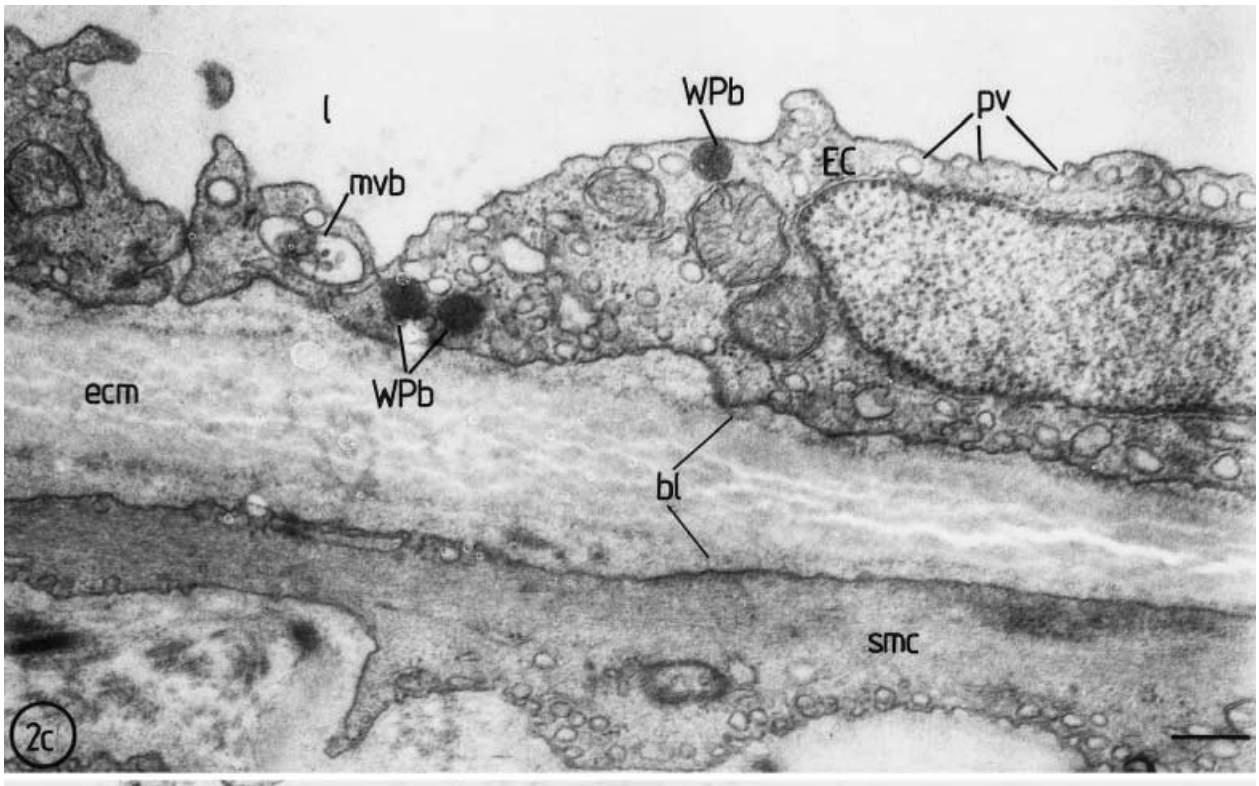


Fig. 2a–d Representative electron micrographs of the structure of the mesenteric resistance arteries. **a** HD group: endothelial cells (*EC*) contain numerous of Weibel-Palade bodies (*WPb*), plasmalemmal vesicles (*pv*), biosynthetic organelles, and protrusions of the abluminal plasmalemma (*arrowheads*) into the apparently enlarged subendothelium enriched in collagen fibers (*c*) and elastin (*e*). **b** HD group: smooth muscle cells (*smc*) are endowed with numerous sarcolemmal vesicles (*sv*) and are surrounded by an unusually thick extracellular matrix (*ecm*) containing abundant collagen fibers (*c*) and frequent calcification centers (*cc*). **c** HD + L-arg group: endothelial cells contain a multivesicular body (*mvb*, a degradative endocytotic compartment), Weibel-Palade bodies (*WPb*), and a thick subendothelial extracellular matrix (*ecm*). **d** HD + L-arg group: the smooth muscle cells (*smc*) produce the surrounding augmented extracellular matrix (*ecm*) that appear expanded, and containing disperse calcification centers (*cc*). (*l* Vascular lumen, *bl* basal lamina.) *Scale bars* **a** 0.29 µm; **b** 0.46 µm; **c** 0.22 µm; **d** 0.38 µm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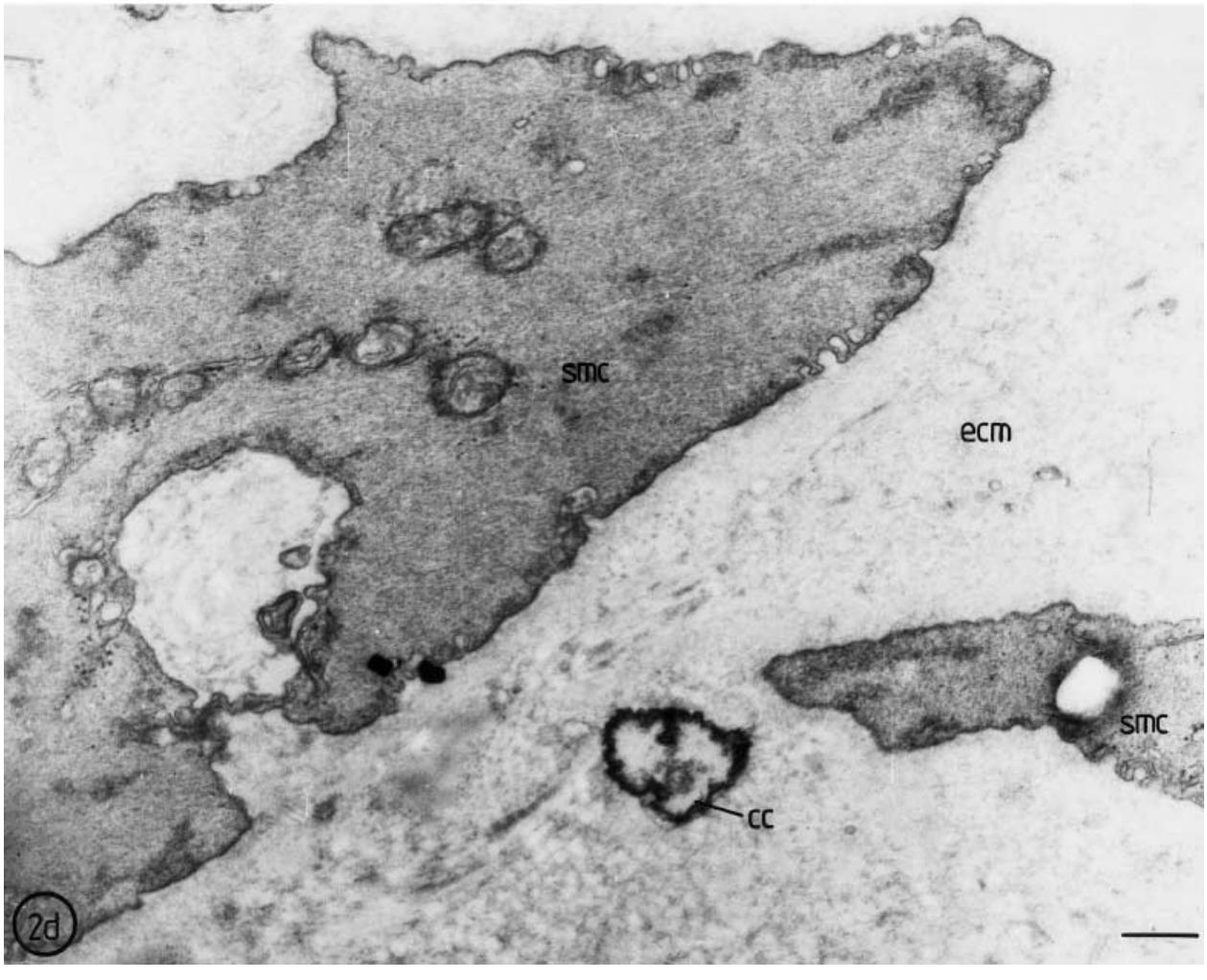
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sensitivity of the response to ACh (expressed as pD_2) in the C and HD ± L-arg groups. Inhibition of NOS by 10^{-4} M L-NAME supplemented to the organ bath reduced the relaxation of the arteries in the C group from 80.20±3.20% to 63.98±2.80%. Arteries in the HD group exposed to L-NAME relaxed to 44.98±3%. In the HD + L-arg group, the relaxation recorded in the presence of L-NAME was 53.16±3.5%.

The vasodilation of arteries to the endothelium-independent NO donor SNP (10^{-8} to 10^{-4} M) was similar in the C and HD ± L-arg groups (Fig. 1b). The maximum relaxation attained at 10^{-4} M SNP was 74.03±1.96% in the C group, 73.74±1.92% in the HD group, and 78.60±0.36% in the HD + L-arg group. There was less sensitivity of the response to SNP in the HD ± L-arg groups versus C group.

The ultrastructure of the resistance arteries

The endothelium of the resistance arteries in the HD group showed a secretory phenotype, numerous copies of Weibel-Palade bodies/cell (source of von Willebrand factor), and an enlarged subendothelial extracellular matrix (Fig. 2a). The smooth muscle cells shifted in the HD group from the contractile to the secretory phenotype, producing a thickened extracellular matrix that embedded frequent calcification centers (Fig. 2b). In the HD + L-arg group, the Weibel-Palade bodies were still of common occurrence in the endothelium, and the subendothelial matrix, rich in collagen bundles, was focally enlarged up to ~1.85-fold (vs C group), as assessed by the morphometric measurements (Fig. 2c). In the HD + L-arg group, a slight diminishment was observed in the thickness of the extracellular matrix which apparently separated adjacent smooth muscle cells (~1.32-fold vs HD group), along with the occurrence of calcification centers (Fig. 2d).

Effect of L-arginine on the heart ultrastructure

Ventricular myocardium

Compared with the C group, where the heart to body weight index was found to be 0.449±0.014, in the HD group this ratio was 0.511±0.01, indicative of left ventricular wall hypertrophy (Table 1). In the latter group, a diminished heart cavity was observed, along with structural modifications of the myocardium consisting of less-organized contractile fibers, frequent interruption of Z-bands, and the occurrence of rich collagen deposits (indicative of interstitial fibrosis; Fig. 3a). L-Arginine administration in the HD group contributed to an enlargement of the ventricular cavity and to a ~16% diminishment of the heart to body weight index, which measured 0.472±0.024 (Table 1). The electron-microscopic examination of the ventricular myocardium in the HD + L-arg group showed an apparently normal structure (Fig. 3b).

Capillaries

In the HD group focal enlargements of the pericapillary extracellular matrix (482.01±45.50 nm) were measured by morphometric analysis, along with the partial narrowing or even collapse of myocardial capillaries (Fig. 4a). After L-arginine administration, restoration of the normal, round contour of the capillaries occurred, and a diminished pericapillary extracellular matrix (172.23± 3.24 nm) was measured (Fig. 4b). The latter value is close to that recorded in the C group, i.e., 158.72± 9.10 nm.

Effect of L-arginine on the structure of the kidney glomeruli

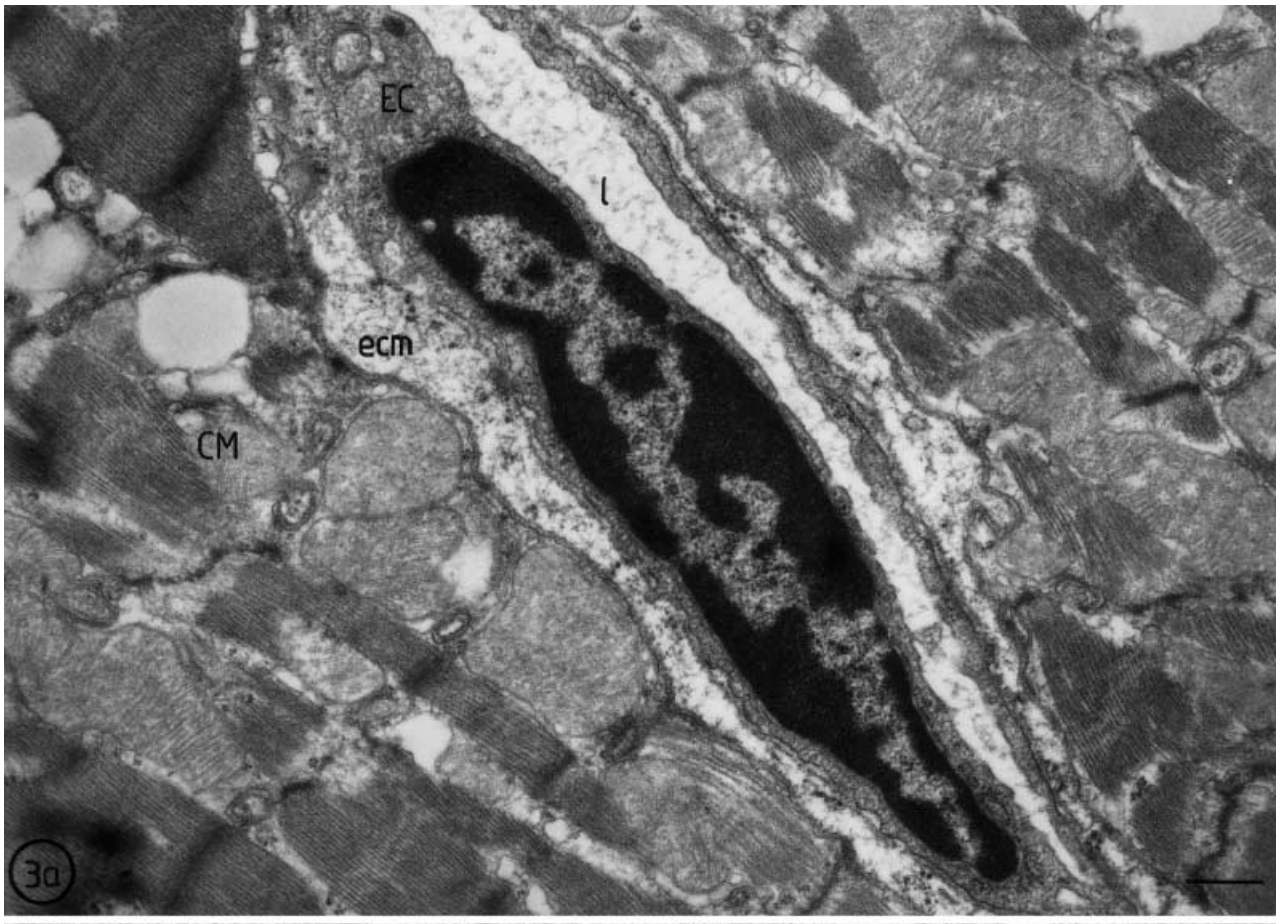
Compared with controls, thickening of the capillary basal lamina was of constant occurrence in the glomerular capillaries of HD group (Fig. 5). Morphometric measurements showed that in some areas the basal lamina was ~30% thickened as compared to the values found in the C group (116±11 nm). In addition to this, in many locations the basal lamina became endowed with polymorphic intercalated nodules (Fig. 5a). Administration of L-arginine in the HD hamsters did not improve either the thickening or the nodular aspect of the kidney capillary basal lamina (Fig. 5b).

Discussion

This study demonstrates that oral supplementation of L-arginine to simultaneous hyperlipemic-diabetic hamsters has distinct effects which depend on the vascular bed, i.e., beneficial effects for heart capillaries and for the vasodilation of the resistance arteries, but no influence on the altered structure of kidney glomerular capillaries. Another finding is that endothelial dysfunction installed in simultaneous hyperlipemia-hyperglycemia (induced in hamsters, for 24 weeks) is not a permanent defect (or there is a reversible stage of the dysfunction), since it appears to be reversible by L-arginine administration in vivo. These results may be important for understanding the altered mechanism(s) of diabetes complicated with accelerated atherosclerosis in humans, and their improvement using L-arginine, the substrate for NO synthesis.

The hamsters were fed daily with 622.14 mg/kg L-arginine, a dose close to 600 mg/kg used in the litera-

Fig. 3a, b The ultrastructure of the ventricular myocardium. ▶ **a** The myocardium of hamsters in the HD group show disarrays of the contractile fibers, frequent interruptions of Z-bands, and accumulation of extracellular matrix indicative of interstitial fibrosis. Note the narrowed contour of the capillary that displays focal enlargements of subendothelial extracellular matrix (*ecm*). **b** The apparently normal structure of the ventricular myocardium in the HD + L-arg group. (*l* Vascular lumen, *EC* endothelial cell, *CM* cardiomyocyte, *m* mitochondria.) *Scale bars* **a** 0. 48 µm; **b** 0.25 µm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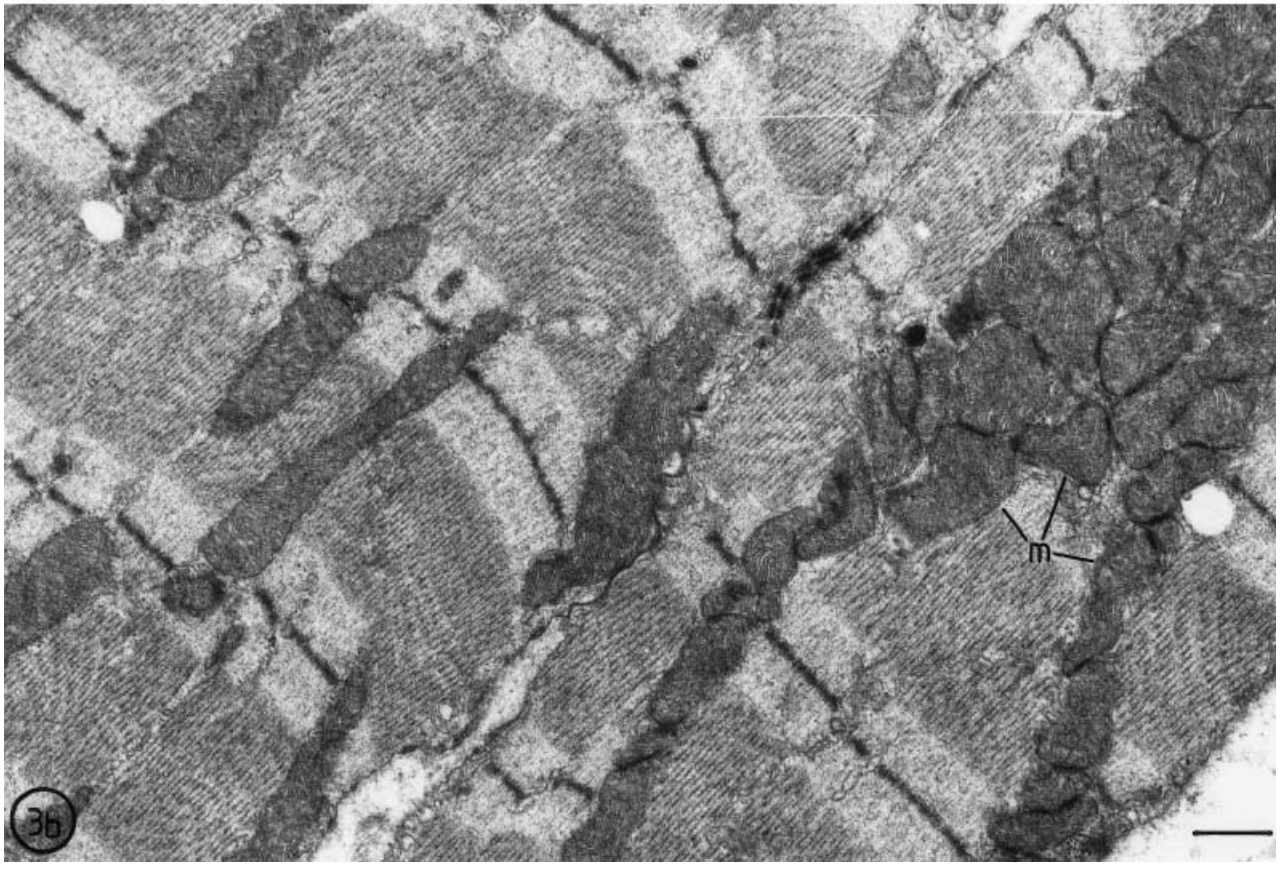
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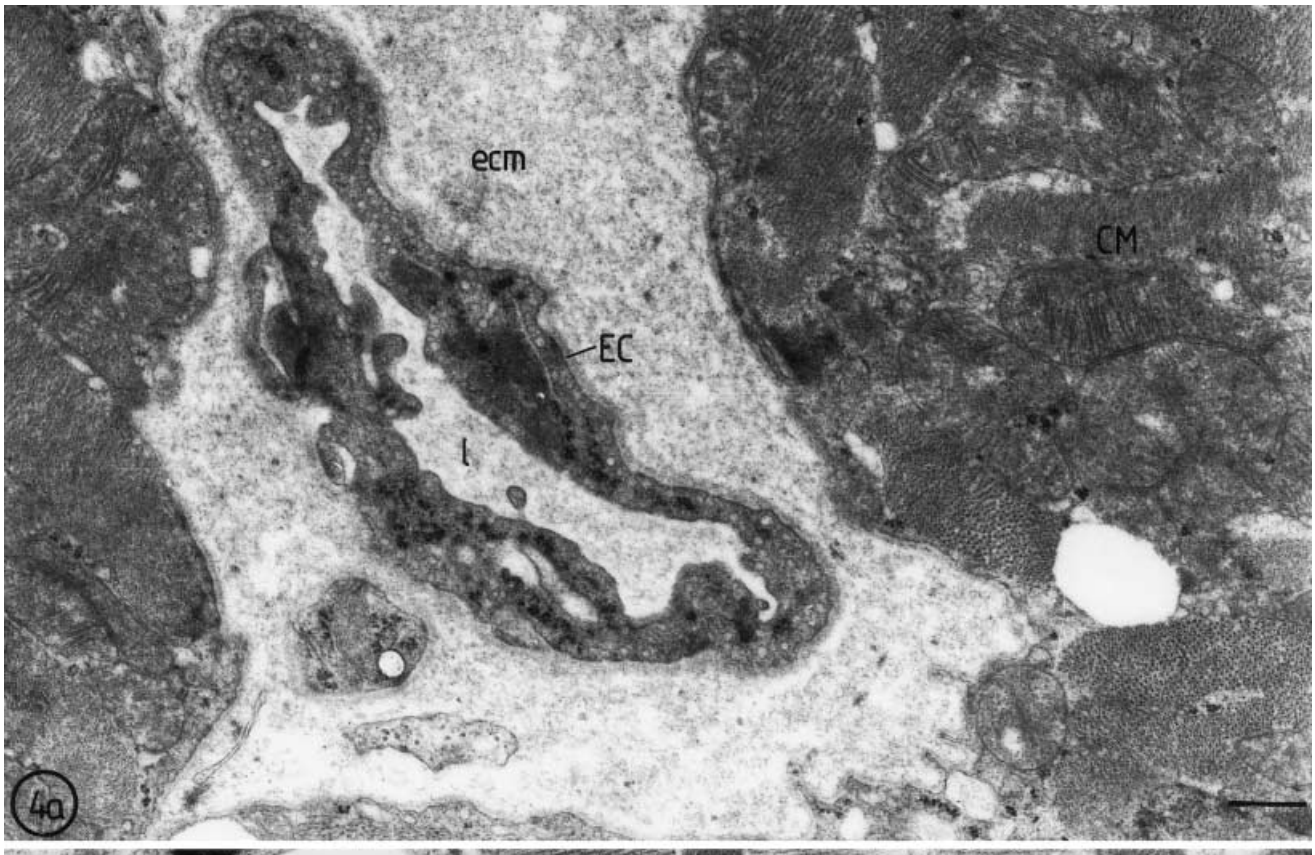

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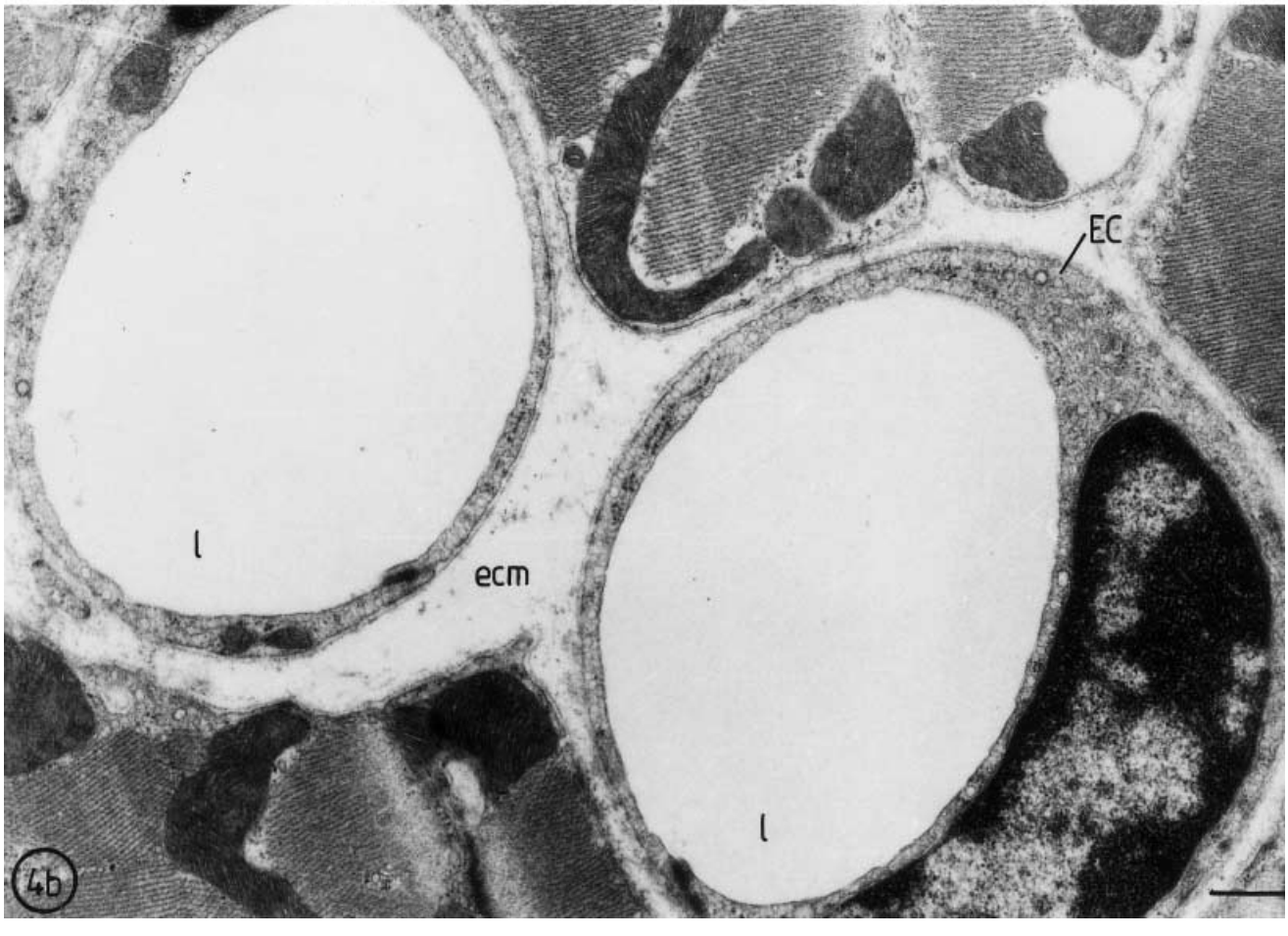

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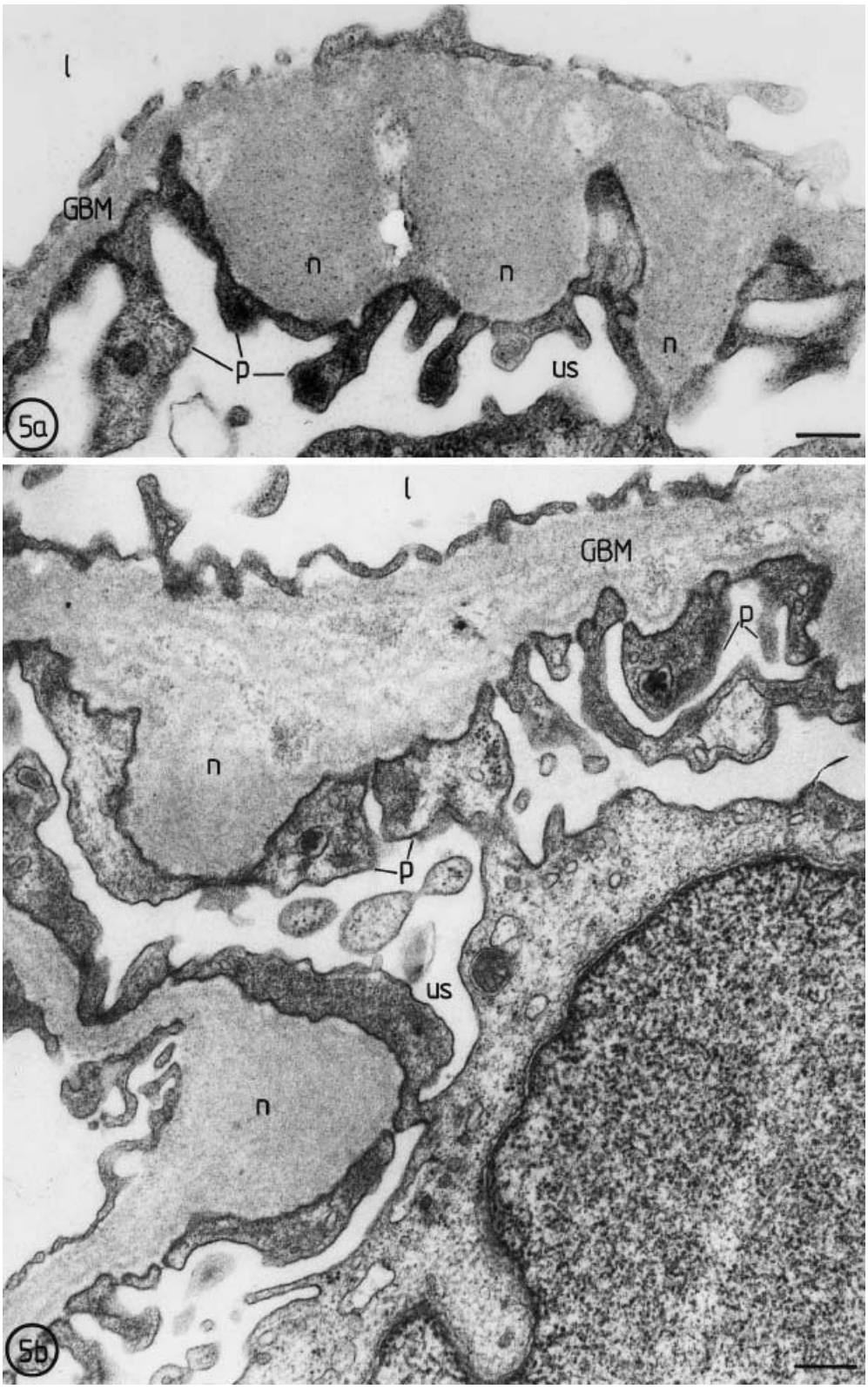

Fig. 5a, b The ultrastructure of the kidney glomerular capillaries. **a** HD group: the glomerular basement membrane (*GBM*) was remarkably thick and displayed numerous polymorphic nodules (*n*) made of extracellular matrix. **b** HD + L-arg group: the same aspect of GBM as in the HD group. (*l* vascular lumen, *P* podocyte, *us* urinary space.) *Scale bars* **a, b** 0.48 µm

◀ **Fig. 4a, b** The ultrastructure of the myocardial capillaries. **a** The capillaries of simultaneous hyperlipemic and diabetic (HD) hamsters (at 24 weeks) turned into narrowed structures, the endothelial cells (*EC*) displayed a secretory phenotype, and the expanded extracellular matrix (*ecm*) contributed to the separation of the capillary from the nearby cardiomyocyte (*CM*). **b** The recovery of the normal round controur of heart capillaries after oral L-arginine administration for 12 weeks (HD + L-arg group). Note the reduced thickness of the subendothelial extracellular matrix to dimensions similar with those found in control (C) group. (*l* Vascular lumen.) *Scale bars* 0.48 µm

ture (Higa et al. 2000; Ohta and Nishida 2001). In simultaneous hyperlipemia and hyperglycemia induced in hamsters, L-arginine supplementation was found to restore glycemia to normal levels. This observation is in agreement with recent data of Mohan and Das (2000) which reported that L-arginine and NO prevent alloxan-induced beta cell damage and the more severe effects of diabetes. The beneficial effect of L-arginine administration on serum glucose levels was also reported for animal models of diabetes and was found to be mediated by polyamine formation (Mendez and Balderas 2001) and/or by an increase in insulin secretion by the pancreatic β-cells that were not damaged after streptozotocin injection (Schmidt et al. 1992).

Another observation was the reduced ACE activity in serum of the HD + L-arg group, a finding that may account for the reversal of the increased blood pressure (Blann and Taberner 1995).

L-Arginine was found to slightly reduce the plasma cholesterol level of HD hamsters. The diminishment of cholesterol concentration by L-arginine was has already been reported for diabetic rats (Mendez and Balderas 2001), cholesterol-fed rabbits (Cooke et al. 1992; Böger et al. 1995), and humans (Böger et al. 1998).

The fragility of erythrocyte membrane to osmotic changes (Jain 1989; Wu et al. 1998) is a biomarker currently used for the evaluation of oxidative processes (Srour et al. 2000) associated with the senescence of red blood cells (Wen et al. 1998), and with storage and gamma irradiation of blood for transfusion purposes (Sharfi et al. 2000). The correlation between osmotic fragility and oxidative stress (produced in diabetes by the reactive oxygen-derived free radicals) relies on peroxidation of cholesterol and linoleic acid molecules of erythrocyte plasmalemma (Inouye et al. 1999a, 1999b, 1999c). Moreover, antioxidants were reported to decrease the osmotic fragility of erythrocytes (Etlik et al. 1997; Kraus et al. 1997). In the hamster experimental model of simultaneous hyperlipemia-hyperglycemia, L-arginine administration resulted in a decrease in the osmotic fragility of erythrocyte plasmalemma, a consequence of decline in lipid peroxidation (Lubec et al. 1997; Ozcelikay et al. 1999). In addition, L-arginine has antioxidant properties due to superoxide scavenging effects (Wascher et al. 1997). It seems that L-arginine administration in the hamster model provides not only an additional supply for NO synthesis, but also an efficient mechanism for superoxide anions scavenging, and for reversing the antioxidant status (Mohan and Das 2000).

Functionally, L-arginine administration improved the impaired endothelial-dependent relaxation of the resistance arteries, similarly to the effects already reported in experimental diabetes (Pieper et al. 1996, 1997). However, in the HD + L-arg group, the vasorelaxation did not reach the control level, possibly due to the rigidity imposed by the calcification centers formed within the vessel wall. Since relaxation to ACh in the C group attained, in the presence of L-NAME, a similar level as in the HD group, one can assume that combined hyperlipemia and diabetes induced a deficit of NO, possibly due to an alteration of NOS activity. In the HD group, blocking of NOS with L-NAME additionally reduced the ACh relaxation, while administration of L-arginine (substrate for NO synthesis) improved the endothelium-dependent vasodilation and therefore had beneficial effects. The favorable effect of L-arginine on the endothelium-dependent relaxation of the resistance arteries can be explained by restoration and/or increase in NO production, by reducing the oxidative stress (Candipan et al. 1996; Böger et al. 1995) and/or by competition with lysine-like residues involved in advanced glycation endproduct (AGE) formation, diminishing the cross-linked vascular AGEs (Pieper 1998).

Hamsters' treatment with L-arginine did not correct the pathological modifications of endothelial cells of the resistance arteries affected by simultaneous hyperlipemia-diabetes. In contrast, aortic endothelial and smooth muscle cells have a mostly normal appearance after L-arginine administration (Sotnikova et al. 2000). The lack of effect of L-arginine on endothelium may be explained by the superimposed hyperlipemia that aggravated the diabetes-induced modifications in the hamster experimental model used. A positive effect of L-arginine was found on the extracellular matrix around smooth muscle cells, which showed a slight diminishment in thickness, a feature that is in agreement with the reduced concentration of AGE-collagen (~35%) measured in the mesentery after in vivo feeding of the hamsters in the HD group with L-arginine (Georgescu and Popov 2000).

In simultaneous hyperlipemia and diabetes, L-arginine supplementation was found to diminish the left ventricular hypertrophy, a finding of interest in the context on recent reports on cardioprotective effects of L-arginine (Suematsu et al. 2001), such as: enhanced myocardial protection during episodes of cardioplegic arrest (Carrier et al. 1998), correction of endothelial dysfunction in chronic heart failure (Hambrecht et al. 2000), prevention of ventricular instability induced by high glucose (D'Amico et al. 2001) and of myocardial fibrosis (Babal et al. 2000), improvement of cardiac performance in congestive heart condition (Bocchi et al. 2000), and the use in myocardial perfusion (Fujita et al. 2000). The cardioprotective effects of L-arginine reported so far are complemented by the results of recent studies emphasizing its efficacy in reduction of mean blood pressure (Okamoto et al. 2001),of monocyte adhesion (Cheng and Balwin 2001), and of platelet aggregation and adhesion (Kaposzta et al. 2001).

L-Arginine administration in hyperlipemic-hyperglycemic hamsters had no positive effect on the renal thick and polymorphic glomerular basement membrane that maintains the nodular structures typical for diabetic glomerulosclerosis (Churg and Sobrin 1982). The higher creatinine levels in plasma of the HD + L-arg group (Table 1) confirm an impeded renal function. Recent results showed that in early diabetic nephropathy an increased NO availability in kidney occurred, as a prereq-

uisite for pathogenesis of glomerular hyperfiltration (Veelken et al. 2000). However, to date the involvement of the L-arginine-NO pathway in renal glomerular disease is still controversial (Klahr 1999). To this contributes the complexity of L-arginine metabolism in kidney (Peters et al. 1999). Findings published so far on both animal models and humans show that additional dietary intake of L-arginine may result in either dose-dependent ameliorating or detrimental consequences in kidney (Peters et al. 1999; Dumont et al. 2001).

In summary, the results of this study provide evidence that endothelial dysfunction in hyperlipemia-hyperglycemia has a reversible stage, which in myocardial capillaries and in the resistance arteries is sensitive to a supplement of NO, provided by oral supplementation of L-arginine, the substrate for NO synthesis.

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