A Selenium Supplement Associated or Not with Vitamin E Delays Early Renal Lesions in Experimental Diabetes in Rats (43976)

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Abstract. **Seventy rats were separated into five groups: one group of 12 was used as a control and received a purified diet, and four groups of streptozotocin-induced diabetic rats, totalling 58, were fed the same diet without or with selenium (Se) supplementation. Of the noncontrol rats, 14 were without supplementation (Group D), 14 were fed a Se-rich yeast diet (i.e., selenion) (Group DSel), 14 received selenomethionine (Group DSm), and 16 received selenomethionine + tocopherol acetate (Group DSmE). Supplementation with Se in all groups was 0.99 μmole/100 g of diet and with tocopherol acetate was 0.145 μmole/100 g. All diabetic rats were mildly balanced by insulin.**

After 24 weeks of diet, plasma glucose tended to decrease in diabetic Se-supplemented groups DSmE > DSm > DSel versus Group D. In DSm and DSmE groups, plasma lipid peroxides also decreased compared with Group D, but this decrease reached significance only for DSmE ($P < 0.01$ for both TBARS and conjugated dienes). Plasma triglycerides also decreased in DSm and DSmE groups versus Group D ($P < 0.01$; $P < 0.05$, respectively). At the same time, Se increased significantly in kidneys of Groups DSel and DSm versus D and more weakly in Group DSmE, but in this case was associated with a large increase of vitamin E. These beneficial effects of selenium supplement and more so of selenium combined with vitamin E were associated with a protection of kidneys in diabetic rats which found expression in a significant correction of renal hyperfiltration ($P < 0.05$) and in a diminution of the number and severity of glomerular lesions ($P < 0.0005$). [P.S.E.B.M. 1996, Vol 211]

Nephropathy remains a major complication of diabetes mellitus and represents the leading cause of chronic renal insufficiency in industrialized countries (1). Because of the human and financial cost of diabetes treatment in terminal renal insufficiency, more investigations to prevent this complication are needed.

The pathogenesis of retinal and renal microangiopathy reflects many factors that may be genetic, hemodynamic, and/or hormonal, but microangiopathy development has been closely related to hyperglycemia (2). Persistent or intermittent hyperglycemia leads to both metabolic and structural alterations which involve an excessive production of free radicals (3). Increased utilization of NADPH *via* the polyol pathway, protein glycosylation, and glucose autooxidation are generators of free radicals (4), which could alter prostaglandin production, thereby increasing the release of thromboxane A2 by the kidneys (5). It has been reported that thromboxane synthetase inhibitors improve glomerular permeability in patients with IDDM

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(6). Similarly, aldose reductase inhibitors in diabetic animal models prevent basement membrane thickening and proteinuria (7). Our laboratory has shown that in diabetic rats antioxidant supplementation modulates platelet hyperactivity (8) and plasma and tissue lipid alterations (9), and can delay cataractogenesis (10).

This work was undertaken to determine whether a selenium (Se) supplementation can delay early glomerular lesions in streptozotocin-induced diabetic rats mildly balanced by insulin. Selenium is a constituent of glutathione peroxidase enzymes which are involved in controlling hydroperoxides and H_2O_2 levels (11). Moreover, selenium has also been associated with insulin-like properties (12) and thus could represent a determining element in the maintenance of oxidative status and metabolic balance in diabetic patients.

Research Design and Methods

Animals. Seventy-six male Sprague Dawley rats with initial body weight of 525 ± 80 g were used for this study. The rats were fed before experimentation with a laboratory chow containing selenium 200 μg/kg of diet and had a normal selenemia of 524 ± 14.5 μg/l. The rats used were relatively old in order to try to accelerate renal lesion development. Twelve rats were used as control (Group C). Sixty-four rats were injected iv with streptozotocin 30 mg/kg dissolved in sodium citrate buffer as already reported (8). Group C was injected with buffer alone. A week after streptozotocin or buffer administration, blood glucose was measured in all animals. All rats with glycemia >2.5 g/l were considered diabetic. Six rats died 48 hr after streptozotocin injection. After another week, all rats received a diet according to the current recommendation for diabetes as already described (8). Briefly, the diet contained 15% (percentage of total calories) proteins, 52% carbohydrates, and 33% lipids. The lipids were a mix of butter, hydrogenated coconut, canola, and sunflower oils (4, 1.7, 7, and 4.5 g/100 g of diet respectively) with a ratio polyunsaturated/saturated fatty acids of 1. Selenium and tocopherol acetate content in purified diet was 20 μg and 5 mg, respectively, for 100 g of diet. Diabetic rats were separated into four groups. Group D (14 rats) received purified diet without supplementation as control group. The other diabetic groups received purified diet supplemented either with Se-rich yeast (i.e., selenion) (14 rats, Group DSel; CelliFe Intern. N°4537; 63.2 mg/100 g of diet), with selenomethionine (14 rats: Group DSm; 194.5 μg/100 g), or with a double supplementation (i.e., selenomethionine + tocopherol acetate) (16 rats; Group DSmE; 194.5 μg/100 g and 62.5 mg/100 g, respectively). The yeast used was a nonrevivable *Saccharomyces cerevisiae* enriched in selenium by fermentation. Selenium in yeast was in the form of 60% selenomethionine and 40% organic selenium compounds. The selenium supplementation (Se) in the three groups was 0.99 μmole of Se element/100 g of diet. Diabetic rats were injected subcutaneously four times per week with 3.2 IU ultra-slow insulin (Novo, Boulogne, France). Body weights of rats were evaluated each week. Urine samples were checked for ketone bodies and proteins with test strips (Rapignost Behring, Marburg, Germany). Fifteen days before sacrifice, 24-hr urines for each rat were collected on metabolic cage and frozen until analysis. After 9 and 24 weeks of diet, blood of each rat was removed from the jugular vein and collected on sodium citrate 3.8%, pH 7.4, after a fast of 7 hr and 24 hr of insulin deprivation. Plasma was isolated by centrifugation and kept at −30°C until analysis. Glycosylated hemoglobin was immediately measured on total blood with a kit (Eagle Diagnostic, Cedar Hill, TX). After blood collection at 24 weeks of diet, rats were sacrificed by iv pentobarbital injection and the kidneys immediately collected.

All animals were housed and used in compliance with National Institute of Health and Medical Research policy on animal care and use similar to the policy expressed by NRC (1985) in its *Guide for the Care and Use of Laboratory Animals*.

Plasma and kidney biochemistry. Glucose, total cholesterol, phospholipids, and triglycerides in plasma were determined by enzymatic kits from BioMérieux (Marcy l'Etoile France) and Merck (Darmstadt Germany). Plasma creatinine was dosed by a colorimetric kit (Sigma Chemical Co., St. Louis, MO). Vitamins A and E in plasma were measured by HPLC (9), and in kidney vitamin E was extracted by the technique of Rittenmaier *et al.* (14) with some modifications already described (9) and measured by HPLC as reported before. Plasma vitamin C was evaluated after protein precipitation by trichloracetic acid according to a colorimetric technique (14). Thiobarbituric acid reactive substances (TBARS) were measured by the technique reported by Dousset *et al.* (15) and conjugated dienes by the technique of Quintanilha (16). Selenium in plasma and kidney was determined by electrothermal atomic absorption spectroscopy with a Varian model Spectra AA 300 apparatus following the technique described by Welz *et al.* (17) with some modifications. Briefly, samples were digested by 65% nitric acid (Ref 100441: Merck, Darmstadt, Germany), and left 12 hr at 60°C. After, samples were diluted with a mix containing 0.4% nitric acid and 0.3% Triton X-100 (Merck) and injected into the graphite furnace with a modifier solution containing copper nitrate 0.5 g/l and magnesium nitrate 1 g/l (1/1 v/v). Absorbance was measured at 196 nm.

Urine biochemistry. Creatinine was dosed with the technique reported before and renal clearance was calculated according to the formula:

$$\text{24-hr Urine Volume} \times \frac{\text{Urine Creatinine mg/l}}{\text{Plasma Creatinine mg/l}}.$$

Albumin concentration in urine was determined by a radial immunodiffusion kit (The Binding Site, Birmingham, United Kingdom).

Histopathology of kidneys. Immediately after collection, two median cross-sections were realized in each kidney of each rat (four sections per rat). The first section was directly fixed in Bouin mix (Sigma, St. Quentin Fallavier, France), the other was frozen at −80°C. On the first section after paraffin embedding, slices of 5 μm were stained either by usual staining (Hematein, Phloxin, Safran) or special staining (Periodic Acid Schiff [PAS]). Standard immunohistochemical techniques were also used, employing commercial IgM and C3 of complement antibodies (Southern Biotech Assoc. Inc., Dako, Carpinteria, CA). Revelation for C3 of complement was performed by a kit L SAB (Streptavidin-biotin; Dako, Carpinteria, CA). On the frozen section, 5 μm slices were realized at −20°C with a cryotome (Microm HM 505 E) for immunofluorescence studies of C3 complement, which was revealed by C3 antibody and IgG marked with fluorescein and observed under microscope ultraviolet light. The study of histopathologic preparations were scored in a double blind fashion, at two different levels of microscope magnification: ×25 for global evaluation and ×250 to observe carefully each glomerula. Lesions were estimated according to two criteria: the extension of deposit and the percentage of glomeruli affected. An index of 1 was attributed to moderate deposit reaching 10%–20% of glomeruli, and an index of 2 for a significant deposit concerning more than 20% of glomeruli. Vitamin E and selenium were determined in kidney as reported before.

Statistical analysis. Unpaired Student's *t* test preceded by analysis of variance (ANOVA) and chi-square test were used to evaluate the significance of differences.

Results

After 24 weeks of diabetes, the weight of the rats was increased by 33% in Group C and only by 15% in Group D ($P < 0.05$ versus Group C), but in selenium-supplemented groups the weight gain reached 18%, 22% and 32%, respectively, for Groups DSel, DSm, and DSmE, and was not significantly different from that of Group C. After 24 weeks, mortality was null for Group C, 6/14 for Group D, and 4/14, 6/14, and 3/16, respectively, for Groups DSel, DSm, and DSmE. Mortality was significantly increased for Groups D and DSm compared with Group C ($P < 0.03$), while not reaching significance for Groups DSel and DSmE. Ketone bodies were not detected during the disease in any rat.

Biochemical plasma parameters are shown in Table I. Results after 9 weeks of diet are reported only when they provided additional information. After 24 weeks of supplementation by selenium in diabetic rats, plasma selenium increased in the three groups DSel, DSm, and DSmE compared with Group C ($P < 0.0005$ for all groups), but only selenomethionine and selenomethionine + vitamin E supplement permitted a significant increase in plasma selenium when compared with Group D, and the double supplementation was the most efficient ($P < 0.05$, $P < 0.0005$, respec-

Table I. Plasma Biochemical Parameters in Control Group (C) and Streptozotocin-Induced Diabetic Rats Fed with an Unsupplemented Purified Diet (D) or Supplemented with Selenion (DSel), Selenomethionine (DSm), or Selenomethionine + Vitamin E (DSmE) for 24 Weeks

	C (n = 12)	D (n = 8)	DSel (n = 10)	DSm (n = 8)	DSmE (n = 13)
Glucose (m*M*)	9.1 ± 0.2	23.0 ± 4.1^{a3}	21.3 ± 4.3^{a2}	19.5 ± 4.0^{a2}	16.7 ± 3.3^{a1}
HbA_1 % 9 weeks		6.3 ± 0.2	6.1 ± 0.2	5.8 ± 0.3	5.5 ± 0.2^{b1,c1}
HbA_1 % 24 weeks	5.0 ± 0.1	6.5 ± 0.1^{a3}	6.4 ± 0.1^{a3}	6.6 ± 0.1^{a3}	6.2 ± 0.1^{a3}
Total cholesterol (m*M*)	1.80 ± 0.1	2.29 ± 0.1^{a2}	2.71 ± 0.3^{a1}	2.40 ± 0.3	2.16 ± 0.2
Phospholipids (m*M*)	1.12 ± 0.1	2.10 ± 0.2^{a3}	2.50 ± 0.4^{a2}	1.76 ± 0.2^{a1}	1.53 ± 0.2^{b1,c1}
Triglycerides (m*M*) 9 weeks		4.88 ± 0.5	3.70 ± 0.76	3.22 ± 0.4^{b1}	3.46 ± 0.5
Triglycerides (m*M*) 24 weeks	2.08 ± 0.2	3.91 ± 0.4^{a3}	4.73 ± 0.1^{a2}	2.37 ± 0.2^{b2}	2.60 ± 0.3^{b1,c1}
Selenium (μ*M*) 9 weeks		6.57 ± 0.4	8.51 ± 0.4^{b2}	10.5 ± 0.3^{b3,c2}	10.6 ± 0.9^{b2}
Selenium (μ*M*) 24 weeks	5.71 ± 0.3	6.72 ± 0.6	8.00 ± 0.4^{a3}	8.34 ± 0.3^{a3,b1}	10.3 ± 0.5^{a3,b3,c2,d2}
Vitamin A (μ*M*)	1.17 ± 0.2	1.19 ± 0.2	1.47 ± 0.10	1.04 ± 0.1^{c1}	1.23 ± 0.1
Vitamin E (μ*M*)	22.8 ± 1.4	35.0 ± 3.5^{a2}	42.0 ± 6.6^{a2}	30.6 ± 4.5	44.4 ± 5.4^{a2}
Vitamin E/TG (m*M* * 10^3)	11.7 ± 1.0	9.44 ± 1	10.2 ± 1.2	12.9 ± 1.6	18.3 ± 1.8^{a2,b2,c2,d1}
Vitamin C (μ*M*)	7.59 ± 0.7	7.27 ± 0.9	8.61 ± 1.5	6.49 ± 1.0	5.92 ± 0.3^{a1}
TBARS (μ*M*)	3.52 ± 0.1	4.73 ± 0.2^{a3}	4.21 ± 0.58	4.60 ± 0.4^{a2}	3.89 ± 0.1^{b2,d1}
Conjugated dienes (μ*M*)	64.0 ± 5.7	127 ± 15.1^{a3}	167 ± 28.4^{a2}	92.2 ± 6.5^{a2,c1}	77.8 ± 7.0^{b2,c2}

Note. Results are mean ± SEM for 8–13 experiments. TG, triglycerides.
Versus C: $^{a1}P < 0.05$; $^{a2}P < 0.01$; $^{a3}P < 0.0005$. Versus D: $^{b1}P < 0.05$; $^{b2}P < 0.01$; $^{b3}P < 0.0005$. Versus DSel: $^{c1}P < 0.05$; $^{c2}P < 0.01$. Versus DSm: $^{d1}P < 0.05$; $^{d2}P < 0.01$.

tively). Glycemia was significantly increased after 24 weeks of diet and of disease in all diabetic groups compared with control ($P < 0.0005$; $P < 0.005$; $P < 0.005$; and $P < 0.05$ for groups D, DSel, DSm, and DSmE, respectively, versus Group C). Glycosylated hemoglobin (total HbA) rates after 24 weeks of disease were significantly augmented in all diabetic groups compared with control group. However, this increase was faster with regard to disease in Group D and DSel, because after only 9 weeks the enhancement of total HbA was almost equivalent to that observed after 24 weeks while after 9 weeks in Groups DSm and DSmE the rates of total Hb glycosylated were significantly lower than those observed after 24 weeks ($P < 0.05$, $P < 0.01$ respectively). Concerning plasma lipids, significant increases of total cholesterol (CHOL), phospholipids (PL), and triglycerides (TG) were observed in Group D compared with Group C ($P < 0.01$; $P < 0.0005$; and $P < 0.0005$ for CHOL, PL, and TG, respectively). Although slighter, an increase of these lipids was also observed in Group DSel compared with Group C ($P < 0.05$; $P < 0.01$; and $P < 0.01$ for CHOL, PL, and TG, respectively). Moreover, it is interesting to remark that significant decreases of triglycerides appeared in Groups DSm and DsmE compared with Group D ($P < 0.01$; $P < 0.05$, respectively) after 24 weeks of diet, but also after 9 weeks ($P < 0.05$; $P < 0.01$). Phospholipids were also significantly decreased in Group DSmE compared with Group D after 24 weeks of diet ($P < 0.01$).

Concerning antioxidant vitamins, a weak increase of plasma vitamin A was observed in the group supplemented with selenium-rich yeast. Vitamin E was increased in Groups D, DSel, and DSmE compared with Group C ($P < 0.01$ for all groups). But when the ratio of vitamin E to triglycerides was calculated, it tended to decrease in Groups D and DSel compared with control, and on the contrary increased in Groups DSm and DSmE, significantly so in the latter ($P < 0.01$). Plasma vitamin C rates were not changed in diabetic groups compared with Group C, except for Group DSmE, where a significant decrease was noted ($P < 0.05$). Oxidation markers in plasma were evaluated by dosage of thiobarbituric reactive substances (TBARS), and by the measurement of conjugated dienes. A significant increase in TBARS and conjugated dienes was observed in Group D compared with Group C after 24 weeks of diet ($P < 0.0005$). Selenomethionine + vitamin E supplementation cancelled this increase in conjugated dienes and TBARS. In Group DSel, TBARS after 24 weeks of diet were not significantly increased compared with Group C, and conjugated dienes tended to decrease in Group DSm compared with Group D at both 9 and 24 weeks.

Renal parameters are indicated in Table II. Weight of kidneys was significantly increased in all diabetic groups compared with Group C ($P < 0.01$). Similarly, water consumed and 24-hr urine volume were increased in diabetic rats compared with control. However, it is interesting to note that these volumes tended to diminish with selenium supplementation, and in DSmE group, 24-hr urine volume significantly decreased compared with Group D ($P < 0.05$). Urine microalbuminuria excretion was increased in all diabetic groups compared with Group C ($P < 0.05$). A large variability was observed in this parameter among the rats. Creatinine renal clearance was significantly increased (50%) in group D compared with Group C ($P < 0.005$), while it remained unchanged in all other diabetic groups supplemented with selenium.

Renal lesions observed (eosinophil, positive PAS deposits) were those classically seen in diabetic nephropathies. Glomerular lesions observed in diabetic groups remained very discrete, and vascular or interstitial lesions were exceptional, being observed only in two rats of Group D. Four sorts of lesions were observed: (i) a diffuse glomerulosclerosis represented by eosinophil and positive PAS lamellar deposits on mesangial axis (Fig. 1A); (ii) nodular lesions of the same composition but in peripheral or intercapillary position

Table II. Kidney Parameters in Control Group (C) and Streptozotocin-Induced Diabetic Rats Fed with an Unsupplemented Purified Diet (D) or Supplemented with Selenion (DSel), Selenomethionine (DSm), or Selenomethionine + Vitamin E (DSmE) for 24 Weeks

	C ($n = 12$)	D ($n = 7$	DSel ($n = 9$)	DSm ($n = 7$	DSmE ($n = 11$)
2 kidneys weight (g)	4.32 ± 0.1	5.28 ± 0.3^{a2}	5.14 ± 0.1^{a3}	5.24 ± 0.1^{a3}	5.12 ± 0.3^{a2}
Selenium (nmol/g dry tissue)	73.2 ± 3.6	72.1 ± 5.5	111 ± 6.7^{a3,b2}	122 ± 5.9^{a3,b3}	86 ± 5.9^{c1,d2}
Vitamin A (nmol/g dry tissue)	28.7 ± 4.3	23.5 ± 1.7	22.1 ± 1.9	22.4 ± 2.3	20.8 ± 1.5
Vitamin E (nmol/g dry tissue)	162 ± 16	137 ± 15.3	126 ± 7	142 ± 16.9	172 ± 18.8^{c1}
Water intake (ml/24 hr)	29.7 ± 2.3	83.3 ± 12.8^{a3}	65.4 ± 8.2^{a3}	62.7 ± 3^{a3}	59.6 ± 8.8^{a2}
Urine volume (ml/24 hr)	13.3 ± 2.6	58.9 ± 15.1^{a2}	41.9 ± 8.5^{a2}	49.1 ± 5.3^{a3}	29.9 ± 4.4^{a2,b1}
Urine albumine excretion (mg/24 hr)	2.8 ± 1.5	11.1 ± 3.5^{a1}	36.4 ± 15.6^{a1}	16.3 ± 7.3^{a1}	10.9 ± 3.2^{a1}
Creatinine clearance (ml/sec * 10^{-2})	4.12 ± 0.6	8.41 ± 1.8^{a1}	3.65 ± 0.6^{b1}	4.40 ± 0.7	5.02 ± 0.6^{b1}

Note. Results are mean ± SEM for 7–12 experiments.
Versus C: $^{a1}P < 0.05$; $^{a2}P < 0.01$; $^{a3}P < 0.0005$. Versus D: $^{b1}P < 0.05$; $^{b2}P < 0.01$; $^{b3}P < 0.0005$. Versus DSel: $^{c1}P < 0.05$. Versus DSm: $^{d2}P < 0.01$.

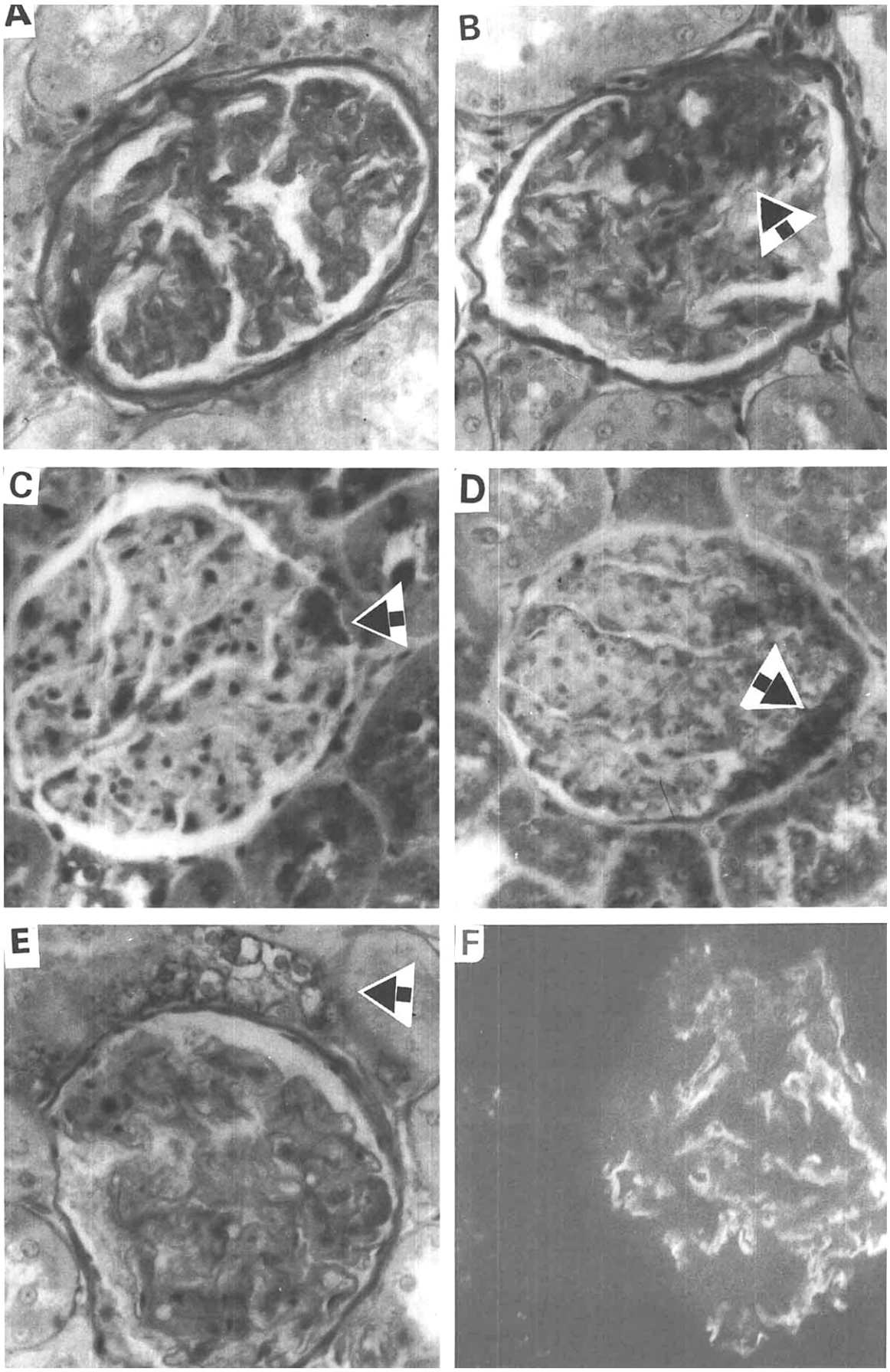

Figure 1. (A) Diffuse glomerulosclerosis: PAS mesangial deposits and irregular thickening of Bowman capsule. (B) Nodular glomerulosclerosis (Kimmelstiel-Wilson lesion) PAS nodules with capillar microaneurysms and irregular thickening of Bowman capsule. (C) Capsular drops and multiple C3 complement deposits in mesangial spaces (Streptavidin-Biotine immunoreaction). (D) C3 complement deposits in crescent against Bowman capsule "fibrin caps" (Streptavidin-Biotine immunoreaction). (E) Armani-Ebstein lesion: PAS glycogen deposits in epithelial cells of proximal tubules. (F) IgM deposits in mesangial spaces (immuno-fluorescence). (A–F) Magnification: ×250.

and more strongly decreasing capillary lumen (Fig. 1B); and (iii and iv) exsudative lesions; fibrin caps (iii) and capsular drops (iv), which were sometimes observed. Fibrin caps were characterized by eosinophil deposits in crescent between the glomerular basal membrane and endothelium. Capsular drops corresponded to an eosinophil round or oval deposit accumulated in Bowman capsule (Fig. 1, C and D). In some cases, tubular lesions were confirmed by glycogen deposits in proximal tubes and plicatures of tubular basal membranes (Fig. 1E). Immunohistochemistry studies, revealing the presence of plasma immunoglobulins in the composition of these deposits, allowed an easier light microscopy reading and also finer screening of lesions as shown in Figure 1, C, D, and F.

The differences observed in lesions among the groups, calculated following the index reported before, are indicated in Figure 2. A significant increase of total lesions ($P < 0.0005$) and of each glomerular or tubular lesion was observed in Group D compared with Group C. However, when diabetic rats were supplemented with selenion, selenomethionine, or selenomethionine + vitamin E, all lesions significantly decreased ($P < 0.0005$). Moreover, for Groups DSm and DSmE some glomerular lesions, more prevalent in advanced nephropathy, disappeared, as did fibrin caps and capsular drops for Group DSmE, and capsular drops for Groups DSm. Similarly, plicatures of tubular membranes representative of serious lesions were not found in these two groups. Briefly, the severity and number of lesions decreased in diabetic rats supplemented with selenium with different treatments compared with diabetic unsupplemented group. Selenomethionine treatment alone or combined with vitamin E was more efficient to prevent lesions than a supplementation afforded by a Se-rich yeast diet. The search for immunoglobulin deposits revealed the presence of C3 deposits along the glomerular basal membranes, which were more predominant in Group D than in Se-supplemented groups.

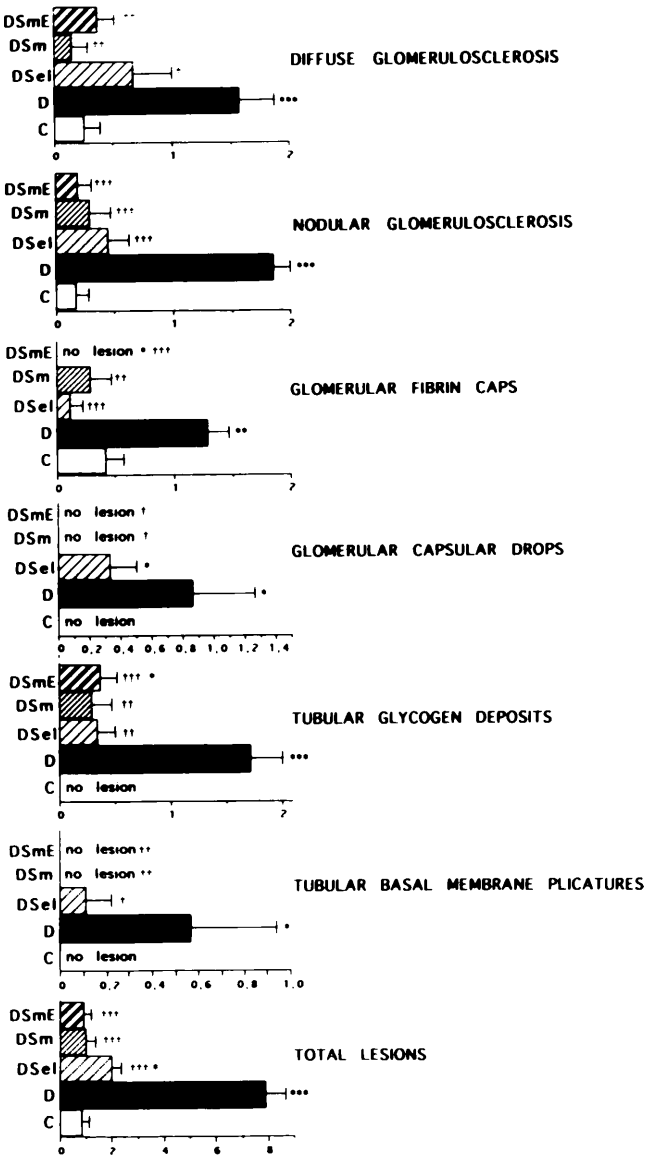

Figure 2. Renal lesion index (number + severity representation) in control group (C) and diabetic groups without supplementation (D) and with selenium supplementation DSel, selenion; DSm, selenomethionine; DSmE, selenomethionine + Vitamin E. Results are mean ± SEM for 7–12 animals. Significance: Versus C: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0005$. Versus D: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.0005$.

Discussion

The goal of this study was to determine whether a nutritional supplement of antioxidant, in reducing free radicals and lipid peroxides, could delay nephropathy in streptozotocin-induced diabetic rats mildly balanced by insulin. Three groups of diabetic rats were supplemented with selenium: either with a Se-rich yeast diet (DSel), selenomethionine (DSm), or with a double supplementation of selenomethionine and vitamin E (DSmE). These treatments permitted an increase of selenium in plasma in the three groups compared with the unsupplemented diabetic group (D) or the control group (C) without disease. It is interesting to note that a double supplementation (DSmE) enhanced plasma selenium more efficiently than did the group with selenomethionine alone. In contrast, in kidney, selenium treatments significantly increased tissue selenium, but this increase was lower in Group DSmE. The regulation of selenium rate is undoubtedly more finely controlled in kidney than in plasma, as already observed in platelets (18). However, antioxidant treatment in diabetic rats modified kidney function. It is known that insulin-dependent diabetes in humans is characterized by an increase of glomerular filtration rate (GFR) and kidney size, which subsists for several years at the *incipiens* stage of disease (19). In our model of diabetic rats mildly balanced by insulin (Group D), we observed both renal hypertrophy and

renal hyperfiltration, the creatinine renal clearance being multiplied by 2.5 in Group D compared with Group C, and kidney weight increased by about 20%. When a selenium supplement was added to the diet, renal clearance was normalized although the hypertrophy persisted. Previous studies suggested that endocrine, nutritional, and/or metabolic manipulations affecting renal function in diabetic rats caused changes both in kidney size and GFR (20). However, it has also been observed that a normalization of GFR by insulin pump therapy in diabetic patients was not accompanied by a reduction of kidney size (21). In our work, this amelioration of kidney function by antioxidant treatment was related to a significant decrease in total glomerular lesions in Groups DSel, DSm, and DSmE compared with Group D ($P < 0.0005$). The mix and the worsening of glomerular lesions during the disease are characteristic of diabetic nephropathy (22). Diffuse glomerulosclerosis with enlargement of mesangium and nodular glomerulosclerosis with capillary compression can be observed in early nephropathy (23). Exudative lesions such as fibrin caps, and especially capsular drops, are more representative of advanced nephropathy (24). It is interesting to note in both Groups DSm and DSmE that exudative lesions were cancelled concerning capsular drops in two groups and also for fibrin caps in Group DSmE. In parallel, in these groups no plicatures of the tubular basal membrane, which are representative of a serious tubular alteration, were observed. Glycogen deposits in proximal tubules were distributed in all diabetic groups, although decreased in Se-supplemented diabetic rats. This lesion has been reported to be rare in human patients (23), but in this animal model seems more frequent, perhaps due to a greater synthesis of glycogen in diabetic rats (25). It is necessary also to note that total and specific glomerular lesions in diabetic groups supplemented with selenomethionine were relatively less numerous than in diabetic rats supplemented with a Se-rich yeast diet, perhaps due to a lesser absorption of selenium when administered as a yeast. We observed after 9 weeks a significant decrease of plasma selenium in group DSel versus DSm ($P < 0.05$) and a relative diminution in kidney selenium in this group compared with DSm after 24 weeks of diet.

Severity of nephropathy has been associated in diabetic patients with an increase in urine microalbuminuria excretion to a rate of >45 mg/24 hr (26). However, in early nephropathy microalbuminuria was found to vary greatly among patients and to be rarely related to renal attacks (27). In this study, we also observed a large variability for this parameter, in diabetic rats. However, an increase of microalbuminuria was noted in all diabetic groups compared with Group C.

The effect of selenium on some biological parameters could be related to its beneficial effect on renal lesions. In diabetic rats treated by selenium, glycemia measured in a hypoinsulinic period (24 hr after injection) tended to decrease in Se-supplemented diabetic groups compared with Group D (unsupplemented). Total HbA1 after 24 weeks of disease was augmented in all diabetic rats compared with control, but after only 9 weeks of disease we observed a lesser increase in Se-treated groups, which became significantly lower in DSm and DSmE compared with the rate observed at 24 weeks ($P < 0.05$). This observation hypothesizes better glycemic control in these groups during the disease. A strict control of glycemia has been associated with a reduction of glomerular lesions (28), and the delaying of lesions observed in this study could be related in part to insulin-like properties of selenium and intensified by those of vitamin E (12, 29), ensuring a decreased requirement for insulin.

Several studies confirm that selenium has an "antioxidant" role (11) and also spares vitamin E and vice versa (30). Moreover, selenomethionine and selenocysteine have been reported to be more efficient than sulfur compounds at decomposing peroxides and could act as free radical scavengers to protect aminoacids and proteins (11). Selenium, through the activity of enzymes, glutathione peroxidaxe (GPXSe), and phopholipid hydroperoxide glutathione peroxidase (PHGPXSe), is essential to maintaining the oxidative status. Diabetes in the rat did not induce a decrease of selenium or vitamin E in plasma and kidney under our diet conditions, but a reduced antioxidant reserve in diabetes has been reported (31), and the need for antioxidant supplementation in order to have a balanced oxidative status in diabetes remains questionable. Effectively, metabolic changes such as the increase of aldose reductase activity, which has been observed in several tissues including the kidney (32), could be in part responsible for the alteration of glomerular permeability (33) and favor oxidative processes involved in glomerulopathy, as in retinopathy. Aldose reductase hyperactivity in using NADPH, decreases reduced glutathione (3). Selenomethionine supplementation could prevent GSH decrease, both by its capacity to react directly with hydroperoxides (11) and by increasing GPXSe and PHGPXSe levels and activities (33, 34). These enzymes catalyze the redox reaction between hydroperoxides and glutathione, allowing prevention of GSH oxidation. Nevertheless, we must note that the rate of reaction catalyzed by selenides is substantially lower than that obtained in the presence of enzymes (11).

We also observed a decrease of mortality in group DSmE and a more relative one in Group DSel perhaps related to vitamin E content of this yeast. Moreover, triglycerides decreased in Groups DSm and DSmE compared with Group D, as already observed with vitamin E supplementation alone (8). Selenium defi-

ciency has been associated with an enhanced synthesis of low and very low density lipoproteins by liver of hypertensive rats (35). The diminution of triglycerides in diabetic rats could be a determining factor in maintaining a lower rate of lipid hydroperoxides (36), thereby contributing to a decreased synthesis of mediators of glomerular injury as TXA2 (5). In Group DSmE, the two plasma oxidative parameters measured, TBARS and conjugated dienes, were significantly decreased compared with Group D after 24 weeks of diet, but only modulated for TBARS in group DSel and conjugated dienes in group DSm. This indicates that the plasma oxidative status was better balanced by a selenium supplementation combined with vitamin E as already observed in rabbits with atherosclerosis (37). Similarly, the combination of selenomethionine + vitamin E increased the ratio of vitamin E to triglycerides and plasma selenium, and caused the total disappearance of advanced glomerular lesions. The combination of vitamin E and selenomethionine, by increasing the effect on the improvement of metabolic processes and perhaps by modulating selenium absorption, permits an optimal effect to delay nephropathy.

In conclusion, selenium supplementation, particularly selenomethionine and selenomethionine + vitamin E, was efficient for preventing glomerular lesions in diabetic rats. This effect could be due in part to better metabolic control but also and perhaps especially to a prevention of oxidative damage *in situ* of renal endothelium, which could be associated with an excessive production of hydroperoxides in this disease (38). Although caution must be exercised in extrapolating data obtained from rats with experimental diabetes to human diabetes, the control of oxidative status by antioxidant treatment in diabetic patients with increased glomerular filtration could be interesting additive therapy to delay nephropathy.

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