INTERNAL MEDICINE GRAND ROUNDS

May 13, 1999

Is the Age-Related Decline in Renal Function Inevitable?

Strategies for the Modulation of Age-Related Glomerulosclerosis, Tubulointerstitial Fibrosis, and Decline in Renal Function

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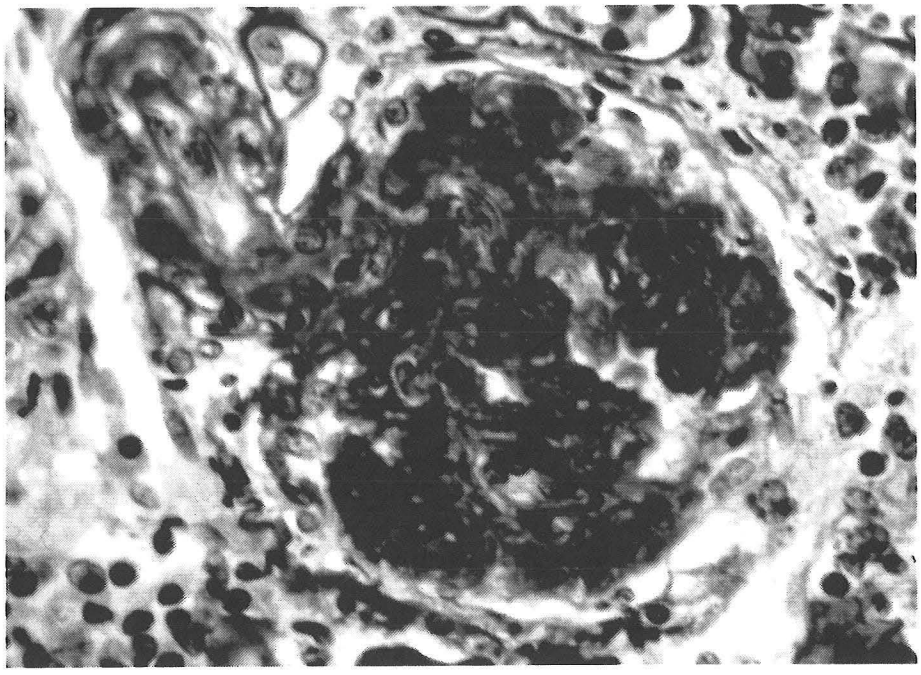

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Research Interests:

1) Regulation of renal phosphate transport

2) Role of cholesterol and glycosphingolipids in regulation of renal function

3) Progression of diabetic and age-related renal disease

4) Clinical applications of near-infrared tissue spectroscopy

INTRODUCTION

The biologic process of aging initiates various structural and functional changes within the kidney. Renal maintenance appears unaffected until internal and external demands such as systemic hemodynamic alterations, intercurrent infections, immunologic processes, drugs, toxins, and other organ system failure intervene and compromise renal function. The aging kidney may be less tolerant of insult and unable to adapt, and therefore predispose the elderly to more significant renal compromise than expected. Increasing prevalence of renal diseases in a growing population of elderly necessitates understanding these anatomic and physiologic changes. Accordingly, this grand rounds attempts to provide a review of our current understanding of the interactions of renal function, aging, and renal disease, and provide potential strategies for the prevention or modulation of age-related renal dysfunction.

RENAL ANATOMY AND STRUCTURE

Renal mass progressively declines with advancing age. Glomerulosclerosis leads to a change in renal weight from 250-270 gm during young adulthood to 180-200 gm by age 90 (1). Postmortem findings of this loss of renal mass can be seen as a decrease in renal size and volume by intravenous urography and CT scanning (2, 3). These changes may be age appropriate as suggested by the Kasiske et al study in which 357 accidental death victims excluded for renal and other comorbid conditions were examined (4). There was little change in renal mass when renal weight was adjusted for a concurrent decrease in body surface area seen with aging (4).

Histologic examination is notable for a decrease in glomeruli number of as much as 30-50% by age 70 (5). Ischemic obsolescence of cortical glomeruli is predominant with relative sparing of the renal medulla. Loss of glomerular tuft lobulation, an 8-12% increase in mesangial volume, and progressive capillary collapse with obliteration of the lumen of the afferent arteriole characterize this histologic obsolescence (6). There is thickening and wrinkling of the basement membranes of both the glomeruli and tubules with eventual reduction and simplification of the vascular channels (7, 8, 9). Juxtamedullary glomeruli see a shunting of blood from the afferent to efferent arterioles resulting in a redistribution of blood flow favoring the renal medulla (10)(Figure 1)

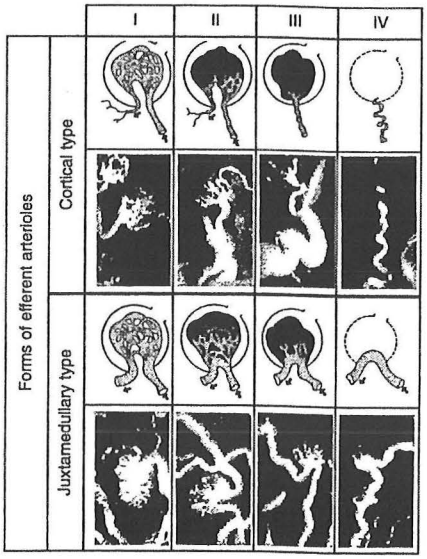


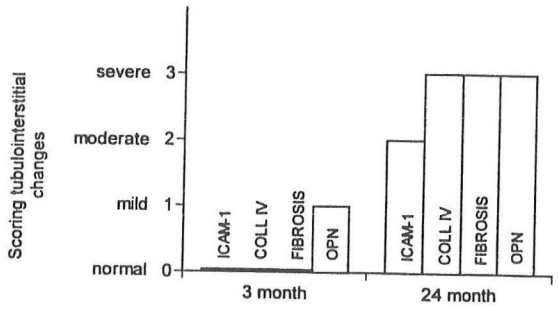
FIGURE 1

Blood flow is maintained to the arteriolar recta vera, the primary vascular supply of the medulla, which are not decreased in number with age (11). Hyaline deposits in residual glomeruli and Bowman's space leaving a scar with little cellular response.

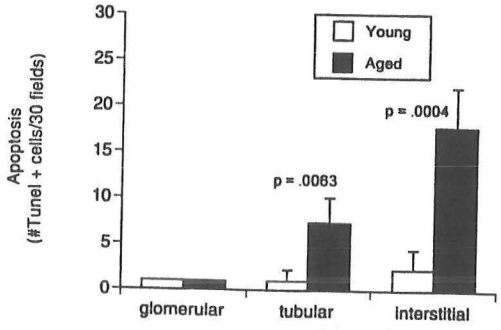
Tubules atrophy, decrease in size and number, and develop distal diverticuli. These diverticuli may collect bacteria or debris. Some have suggested that these outpouchings may contribute to recurrent renal infection or pyelonephritis (12). A recent study has suggested that the tubulointerstitial fibrosis seen in aging may be an active process associated with interstitial inflammation and fibroblast activation with accelerated apoptosis of cells in areas of fibrosis (13). Aged rats show focal tubular cell proliferation, myofibroblast activation, macrophage infiltration, increased immunostaining for the adhesive protein osteopontin and intracellular adhesive molecule-1, and collagen IV deposition (13) (Figure 2).

FIGURE 2

a. Tubulointerstitial Changes in Young and Aged Rats



b. Apoptosis in Young and Aged Rats



This inflammation may be the result of ischemia secondary to peritubular capillary injury and altered endothelial nitric oxide synthase expression (13).

Changes also occur in the intrarenal vasculature with age, independent of hypertension or other renal disease. Normal aging is associated with variable sclerotic changes in the wall of the larger renal vessels, which are made worse in the presence of hypertension (7). Smaller vessels are spared with less than 20% of senescent kidneys from nonhypertensive subjects displaying arteriolar changes (7). Pyelography and angiography of postmortem kidneys of normotensive subjects over age 50 seem to suggest that loss of renal cortical tissue in the elderly may be related more to changes in the renal vasculature than to age alone (14).

RENAL BLOOD FLOW AND GLOMERULAR FILTRATION RATE

Both anatomic and functional changes in renal vasculature appear to contribute to an age related decrease in renal blood flow. Para-aminohippurate clearances drop from 600cc/min/1.73m2 at age 20-29 to 300cc/min/1.73m2 at age 80-89, a change of approximately 10% per decade (15, 16). Xenon washout measurements in 207 healthy potential kidney donors between ages 17-76 demonstrated that this age-related linear decrease in renal blood flow per gram of kidney weight is not uniform within the kidney but that there is a preferential decrease in

cortical blood flow (17). This study is in accord with histologic and functional studies showing a selective loss of cortical vasculature and preservation of medullary flow.

Whether this change in renal plasma flow is affected by possible age related-changes in cardiac output is not clearly established, since some studies have shown an age-related decrease in cardiac output whereas other carefully designed studies have not shown such change (18, 19). There may be a small but definite decrease in the renal fraction of the cardiac output (20). This decrease in renal blood flow to cardiac output may reflect the change in anatomic and vascular responsiveness observed with renal aging.

Renal hemodynamic measurements in aged humans and animals suggest that perhaps an altered functional response of the renal vasculature may be an underlying factor in diminished renal blood flow and increased filtration noted with progressive renal aging. Renal blood flow as measured by PAH clearances increases in response to vasodilatation during infusion of intravenous pyrogen (16) and intraarterial acetylcholine (17) in elderly normotensive human subjects. This vasodilatory response is blunted in the elderly when compared to younger counterparts. When amino acid is infused intravenously, however, GFR and filtration fraction increase while renal plasma flow remains unchanged (21)(Figure 3).

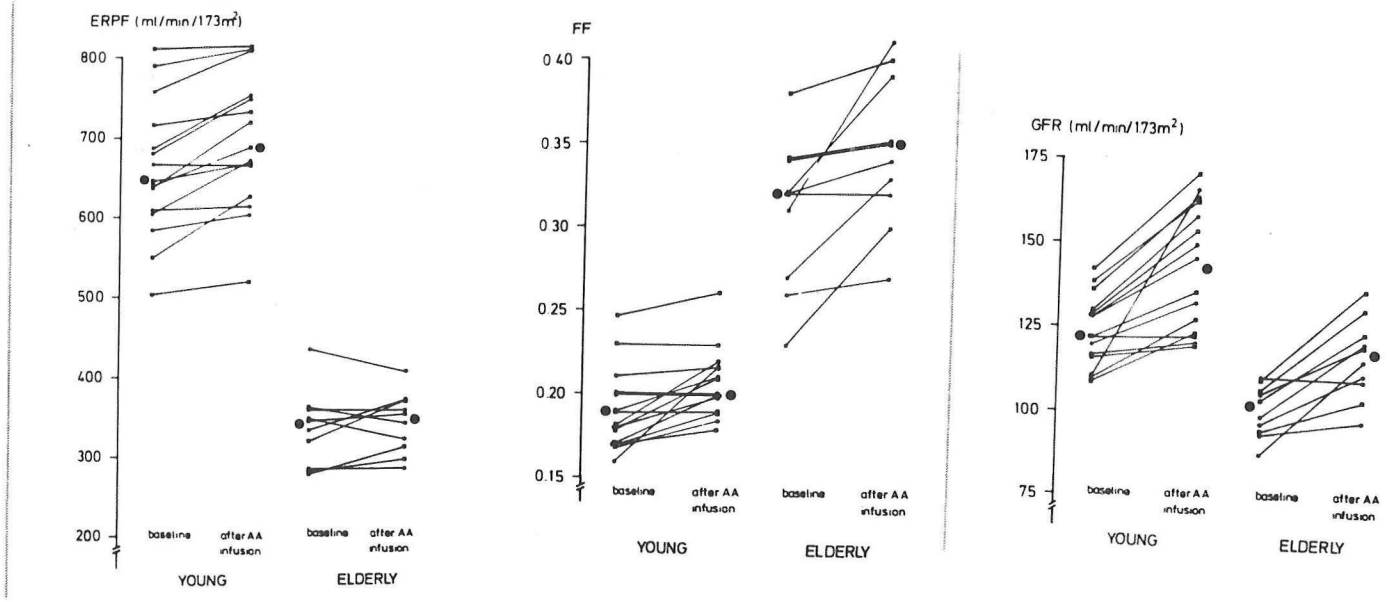


FIGURE 3

The vasoconstrictive response to intraarterial angiotensin is identical in both young and older human subjects (17). A blunted vasodilatory capacity in the face of appropriate vasoconstriction may indicate that the aged kidney may be in a state of renal vasodilatation in order to compensate for underlying sclerotic damage (22). In aged rats there is a marked increase

in the vasoconstrictive response with significant increase in renal vascular resistance (RVR) and decrease in renal plasma flow (RPF) to L-NAME, an L-arginine analogue that competitively inhibits the formation of endothelium derived relaxing factor (EDRF). This suggests a role for possible increased activity of endogenous EDRF in decreasing the vasodilatory reserve observed (23). In vivo micropuncture of young and aged rats also noted no age-related change in the magnitude of the pressor and vasoconstrictive response to angiotensin II infusion. Increased arteriolar resistances of both preglomerular and efferent vessels decreased renal plasma flow and glomerular plasma flow (24). This was accompanied by a rise in the glomerular hydrostatic pressure gradient and increased filtration fraction in both young and older rats (24). However, glomerular capillary ultrafiltration coefficient (K_f) decreased in the older rats leading to a fall in glomerular filtration rate (GFR) and single nephron glomerular filtration rate (SNGFR) (24). In contrast, there was no change in K_f, GFR, or SNGFR in the younger rats (24). Angiotensin II dependent decrease in K_f, via contraction of glomerular mesangial cells, with a subsequentdecrease in filtration surface area, may be a possible explanation for this finding (24).

Despite a linear decrease in renal blood flow with age, filtration fraction appears to increase. Since juxtamedullary nephrons have a higher filtration fraction than cortical nephrons, a combination of preserved medullary flow and decreased renal cortical plasma flow may explain this observation.

Given histologic evidence for nephronal senescence with age, a decline in glomerular filtration rate is expected. However wide variability in the rates of decline in GFR in aging individuals exists depending on methods of measurements, race, gender, genetic variance and other underlying risk factors for renal dysfunction. The decrease in urea clearance with increasing age was noted as early as 1938 by Lewis and Alving (25). Subsequently measurements of GFR by inulin clearances, creatinine clearances, and iothalamate clearances (26) have confirmed the loss of GFR with age. Creatinine clearance drops linearly from 140cc/min/1.73m2 in the third and fourth decade to 97cc/min/1.73m2 by age 80, a rate of decline of 0.8cc/min/1.73m2/yr (27). Iohexol clearances decreased by 1.0cc/min/1.73m2/yr (28). However, one third of 254 healthy elderly subjects followed longitudinally by Lindeman et al. with serial creatinine clearance measurements between 1958 - 1981 were found not to have an absolute change in creatinine clearance measurements (29). Fliser also found inulin clearance studies in normotensive elderly subjects free of hypertension or renal disease and on a normal dietary protein intake of 1.0gm/kg/day to be lower than younger counterparts but slightly greater than 100cc/min/1.73m2 (30). Lew and Bosch also pointed out a drop in GFR with age. At the same time, however, they noted variability of creatinine clearance measurements in relation to protein intake (31). Despite variation in measurement criteria in these studies, it is clear that GFR of aged individuals is lower than younger counterparts. However, this change is not paralleled by a rise in serum creatinine, as muscle mass, from which creatinine is derived, concomitantly decreases with age. Therefore serum creatinine levels generally underestimate GFR in the elderly. The importance of this lies in interpreting clearance during medication dosing and or in assessing risk of the aged kidney to ischemic, toxic, or metabolic events from the serum creatinine alone. Formulas commonly used to estimate GFR can overestimate or underestimate GFR in the elderly (32). Therefore reliability in using these formulas for medication dose adjustment or extrapolating clearance in this population may not be accurate (26). Serial serum drug concentration monitoring may be useful. Some have recommended

routine measurement of creatinine clearances. Again variability in the collection may occur because of diet or other factors. Radionuclide clearance measurements with technetium labelled diethylenetriaminepentacetic acid (^{99}TcDTPA), ^{125}I-iothalamate (GLOFIL), or radiocontrast clearance with single injection iohexol x-ray fluorescence analysis may be considered (26). Currently the best available noninvasive method for accurate determination of GFR in the elderly is the GLOFIL test.

Whether differences in gender have a significant effect on the rate of GFR decline in the aged is not clear. Some have suggested a more gradual decline in females than males while prospective cross-sectional human studies have not borne out this difference.

Racial and genetic differences do seem, however, to affect declining GFR in the elderly. African-American subjects showed a greater declining slope in creatinine clearance with increasing age compared to Caucasians, which in part may result from genetic variance (33). Increased nephrosclerosis was also noted in elderly subjects of Japanese origin versus elderly Caucasians as a cause for worsening renal function (1).

Comparative analysis of GFR, RPF and renovascular resistance between elderly normotensives, hypertensives, and heart failure subjects with the young was notable for worsening hemodynamics and increased RVR in all the elderly groups but especially in those with heart failure (30). Interesting in this study is that elderly hypertensives did not show a significant decline in GFR compared to elderly normotensives. However, when elderly hypertensives not requiring treatment were compared separately with those with a history of drug treatment for hypertension, the latter group had a lower GFR and higher RVR (30). Other studies have also noted age-related renal functional decline in hypertensives (34, 35). Intraglomerular hypertension hastening renal functional impairment in the elderly hypertensive has been suggested in playing a possible role (36). Other risk factors leading to progressive renal dysfunction including atherosclerosis of systemic and renal vasculature, diabetes mellitus, and abnormal lipid metabolism may also play an important role in the functional decline of GFR in the elderly.

RENAL TUBULAR FUNCTION

Anatomic, hemodynamic as well as hormonal changes in the aged kidney impact on crucial physiologic functions, which maintain homeostasis of fluid-electrolytes, acid-base, volume and water balance. Under normal conditions, the aging kidney is able to maintain homeostasis. Under stress however, the adaptive response of the kidney to maintain homeostasis is impaired.

Aging is associated with significant alterations in renal tubular function, including: a) renal concentrating defect (nephrogenic diabetes insipidus), which in the presence of the age-related impairment in thirst results in increased incidence of hypernatremia; b) renal dilution defect, which in the presence of the age-related increase in arginine vasopressin (AVP) levels results in increased incidence of hyponatremia; c) renal acidification defect, which in the presence of age-related hypo-renin and hypo-aldosterone state results in increased incidence of renal tubular acidosis and hyperkalemia; d) renal sodium conservation defect, which results in

increased incidence of volume depletion; e) impairment in renal phosphate transport and inability to conserve phosphate when challenged with a low phosphate diet.

Since the aim of this Medicine Grand Rounds is to explore the pathophysiology of the age-related glomerulosclerosis, tubulointerstial fibrosis and decline in glomerular filtration rate, we will not cover the age-related renal tubular dysfunction in any more detail. This area has been covered in more detail in recent AGING chapters (37, 38 39).

POTENTIAL STRATEGIES FOR THE MODULATION OF AGE-RELATED GLOMERULOSCLEROSIS, TUBULOINTERSTITIAL FIBROSIS, AND DECLINE IN RENAL FUNCTION

Recent evidence indicates that the age-related glomerulosclerosis, tubulointerstitial fibrosis, and decrease in glomerular filtration rate are not necessarily an irreversible consequence of aging. Longitudinal studies conducted by the National Institute of Aging indicate that there are interesting trends in the age-related decline in GFR: a) certain subjects lose more than 2 ml/min of GFR per year; b) certain subjects lose less than 2 ml/min of GFR per year; c) most interestingly, certain other subjects show no decrease in GFR (40, 41)(Figure 4). These very important observations indicate that the age-related decline in GFR is indeed not an irreversible consequence of aging, and that there must be certain factors which initiate the progression of age-related renal disease. Identification of these factors may provide rationale means for the prevention of the age-related decline in renal function.

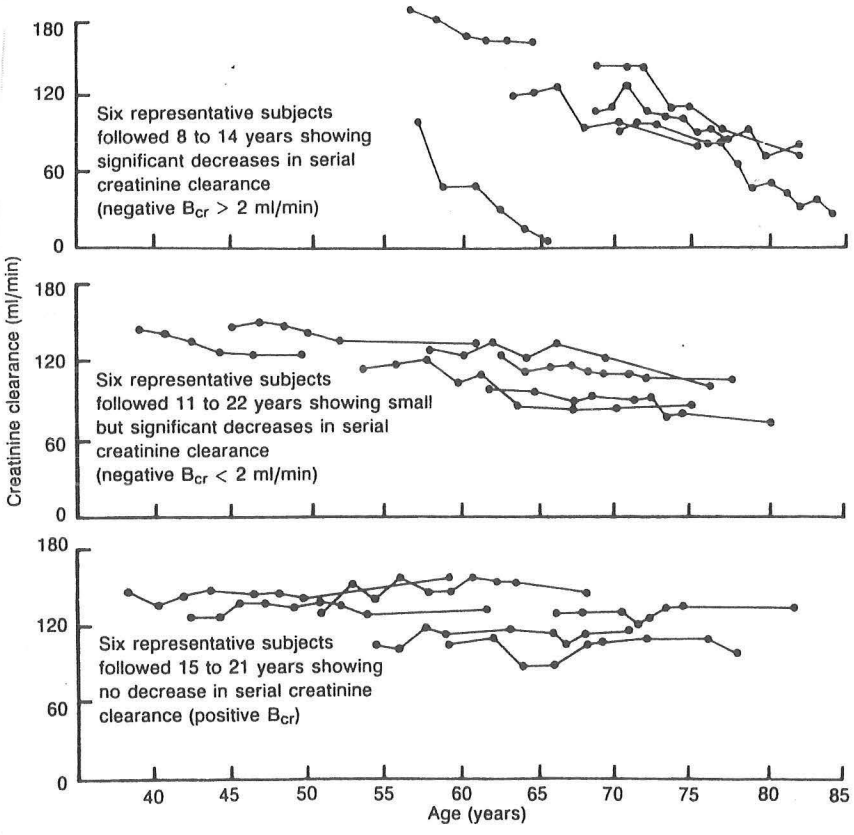
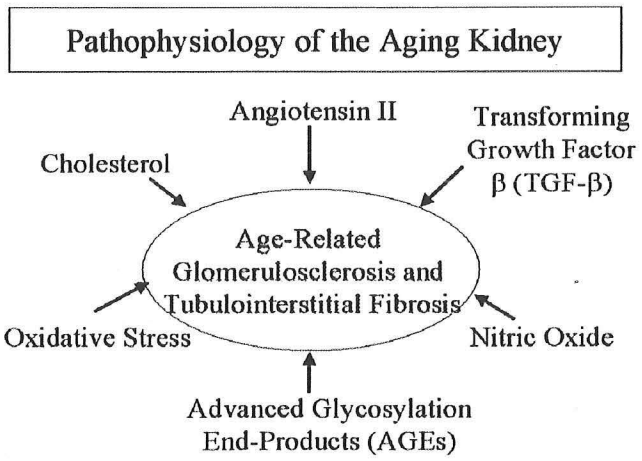


FIGURE 4. Individual plots of serial creatinine clearances versus age in years in representative subjects. One-third of the subjects (92 of 254) showed no decrease in creatinine clearances over the period studied (positive B_{cr} or slope for the creatinine clearance plotted against age in years (41)

Studies mainly conducted in animal models of aging have identified a number of factors that play a role in mediating glomerulosclerosis and tubulointerstitial fibrosis, and modulation of some of these factors result in amelioration of the age-related renal functional and structural alterations. In a way the age-related changes and pathophysiological factors are very similar to those occurring in diabetic nephropathy, except that in diabetes the process is much more accelerated compared to the normal aging process.

FIGURE 5



Role of Angiotensin II

Similar to diabetes and other human and experimental models of progressive nephropathy, a role for angiotensin II in mediating the age-related nephropathy has been demonstrated in aging rats and mice. Several carefully performed studies have shown that long-term treatment with converting enzyme inhibitors (ACEI) resulted in decreases in proteinuria, glomerulosclerosis, and preservation of renal function (42-46).

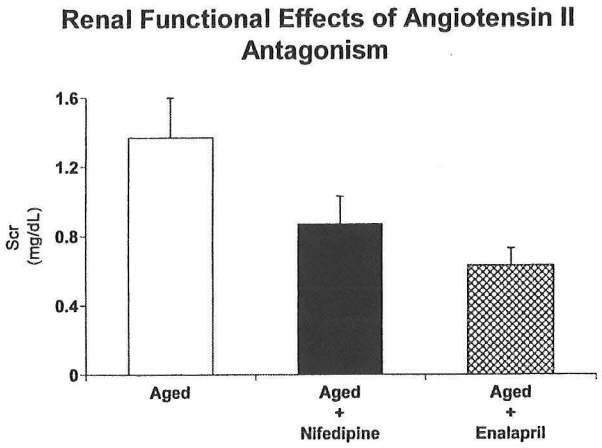


FIGURE 6

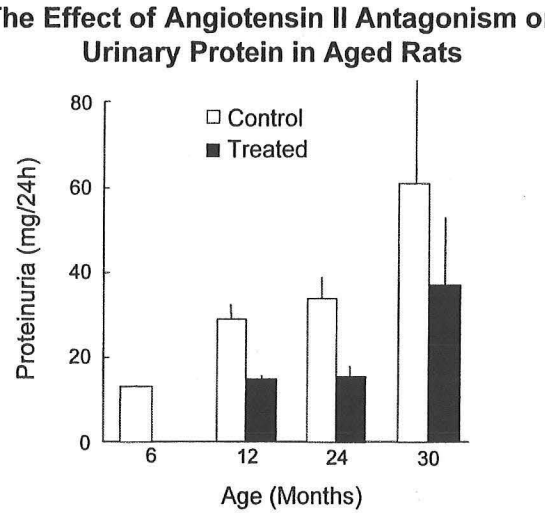


FIGURE 7

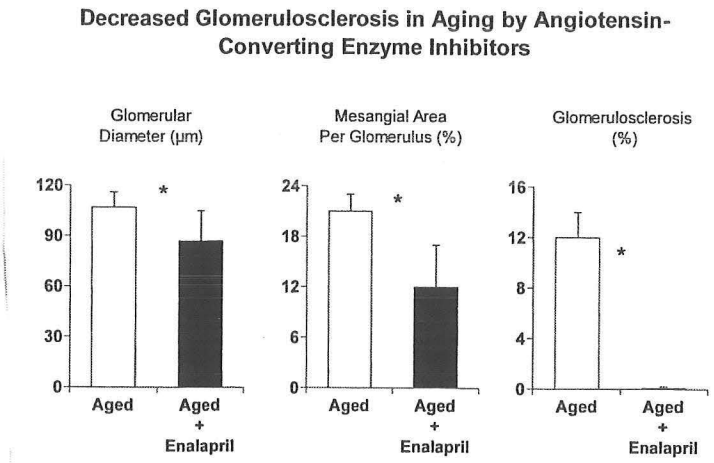


FIGURE 8

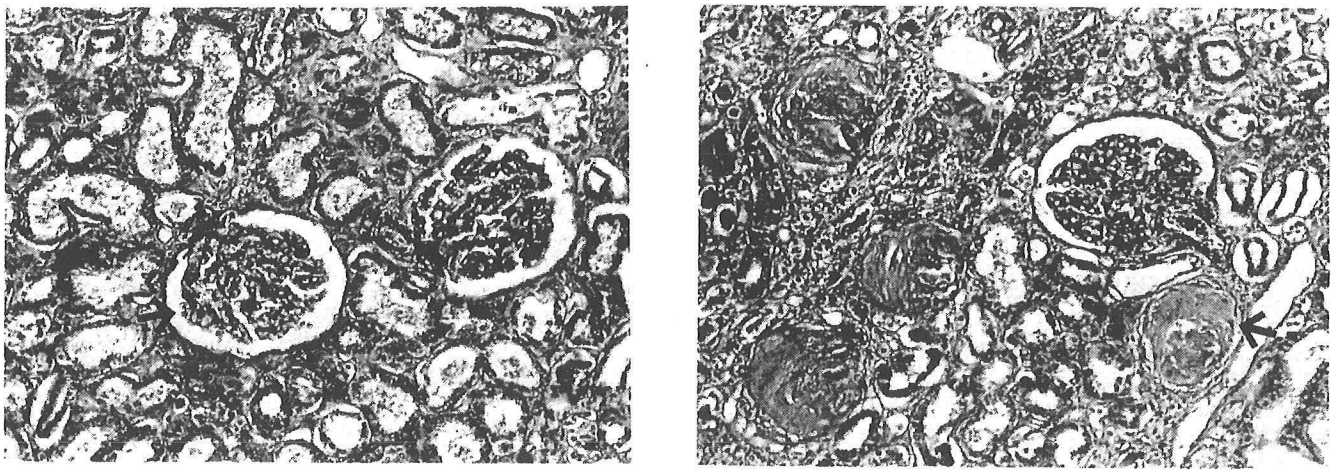


FIGURE 9. (LEFT) Treated animals with conserved glomeruli (arrow). Masson's trichromic, x 100. (RIGHT) Control animal with diffuse glomerulosclerosis (arrow). Masson't trichromic, x 100. (Ferder L, et al. J Am Soc Neph 5:1147-1152, 1994).

Similarly, long-term treatment with ACEI also resulted in significant reduction in tubulointerstitial fibrosis and expression of interstitial alpha-smooth muscle cell-actin (47).

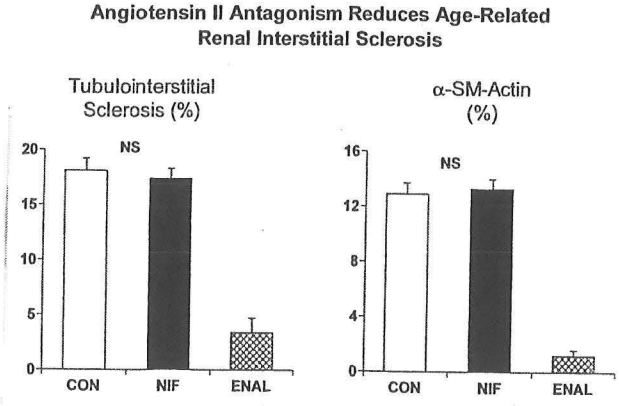


FIGURE 10

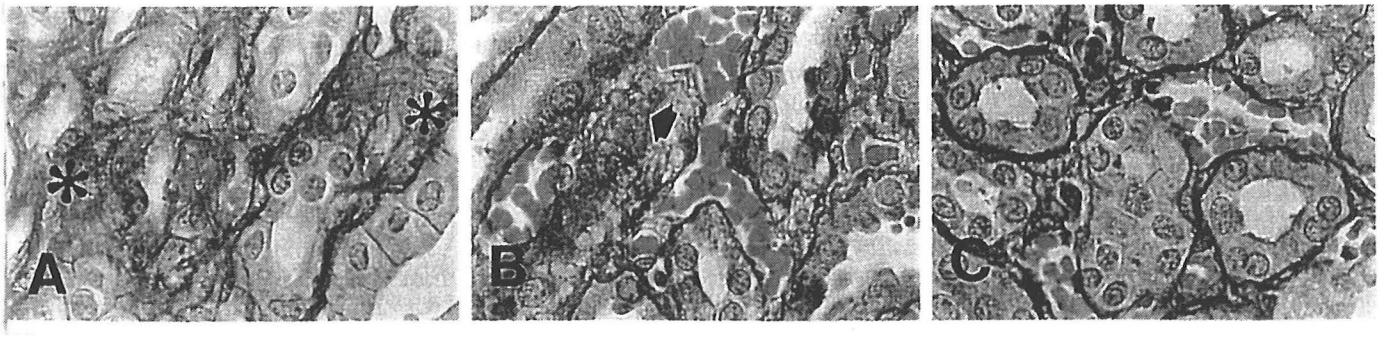


FIGURE 11: (A) Control animal: sclerosis of medullar interstitum (*)(original magnification, x100). (B) Nifedipine-treated animal: sclerosis of medullar interstitum (arrow) (original magnification, x 100). (C) Enalapril-treated animal: normal medullar interstitium (original magnification, x 100). (47)

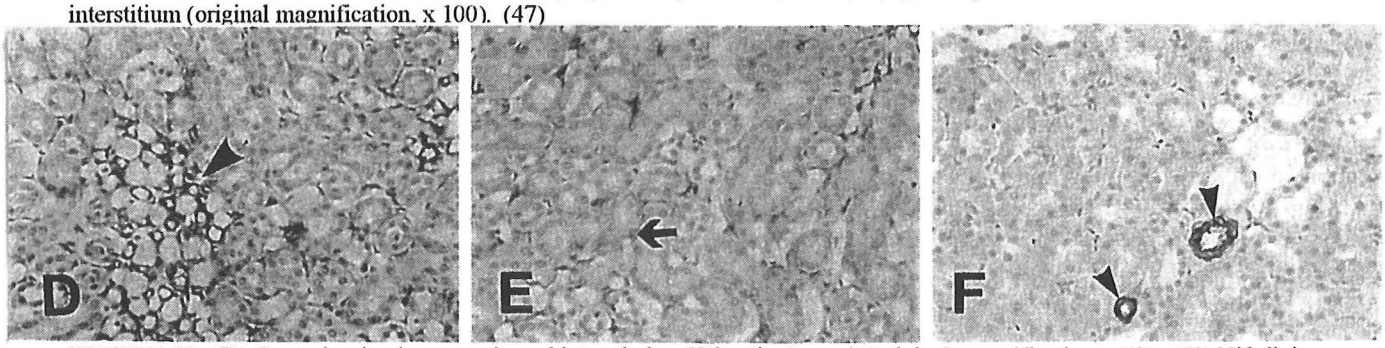
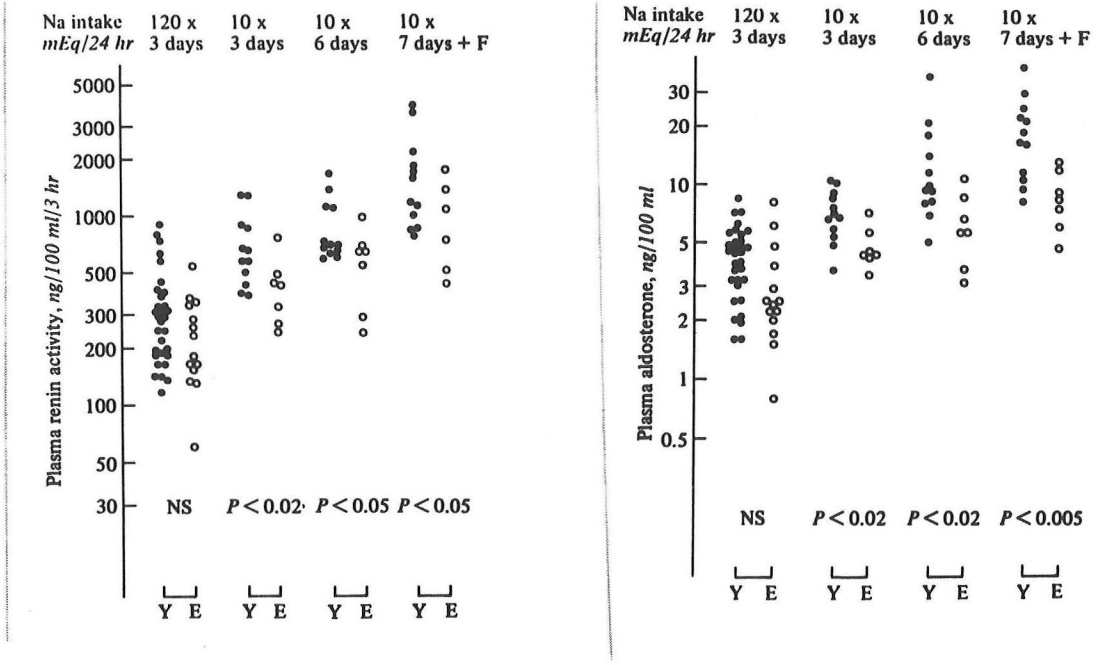


FIGURE 12: (D) Control animal: expression of interstital α-SM-actin (arrow) (original magnification, x70). (E) Nifedipine-treated animal: expression of interstitial α-SM-actin (arrow) (original magnification, x70). (F) Enalapril-treated animal without expression of α-SM-actin in interstitium. It is possible to observe actin in the vessels (arrows)(original magnification, x 70). (47)

The beneficial effects of angiotensin II antagonism are most likely mediated both by hemodynamic and non-hemodynamic mechanisms.

Interestingly, there is a well described age-related decrease in plasma renin and aldosterone concentration, renal renin content and angoitensin II receptor (AT1) abundance (48-64).

FIGURE 13



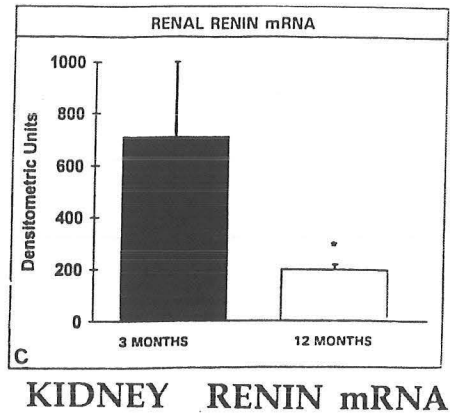


FIGURE 14

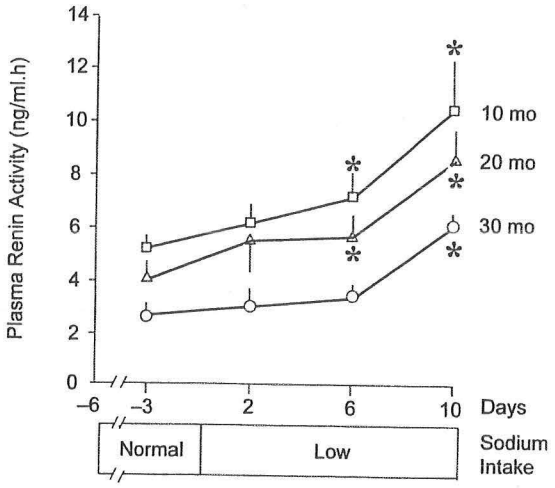


FIGURE 15

Nevertheless, studies in the aged rat suggest that Angiotensin II plays an important role in mediating the age-related decreases in RBF and GFR. In fact, treatment with angiotensin converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) both cause significant increases in RBF and GFR in the aged rats (65).

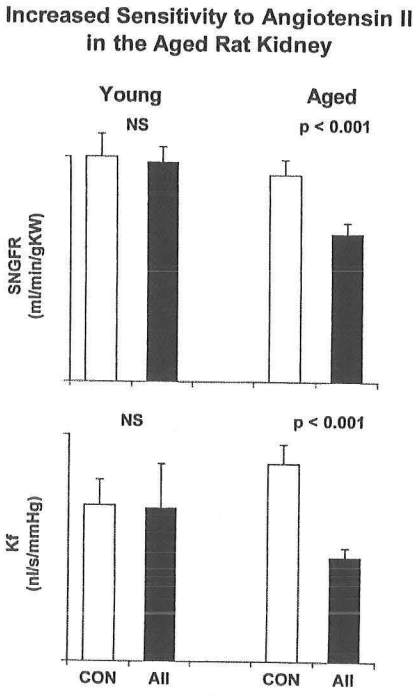


FIGURE 16

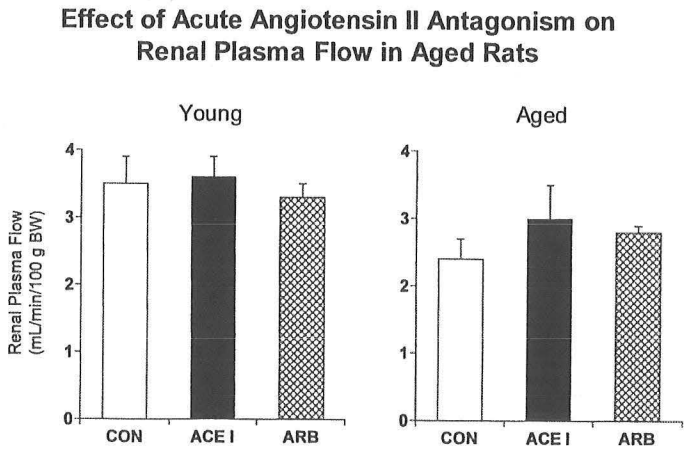


FIGURE 17

The renal protective effects of angiotensin II antagonism can also be mediated by several additional non-hemodynamic mechanisms, including modulation of growth factor expression (especially TGF-β), extracellular matrix protein synthesis and accumulation, recruitment of monocytes and macrophages, and regulation of nitric oxide synthesis (66-72).

FIGURE 18

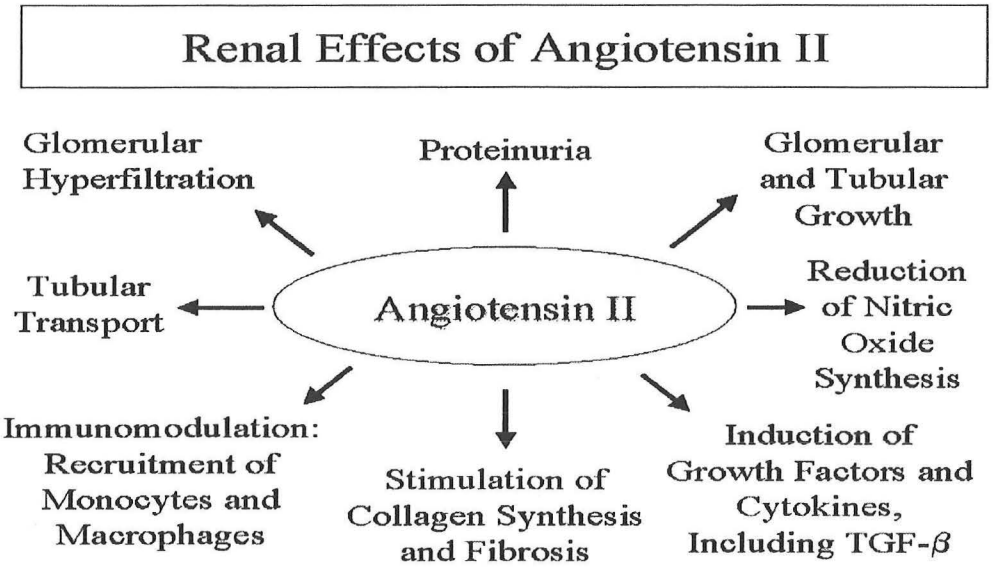
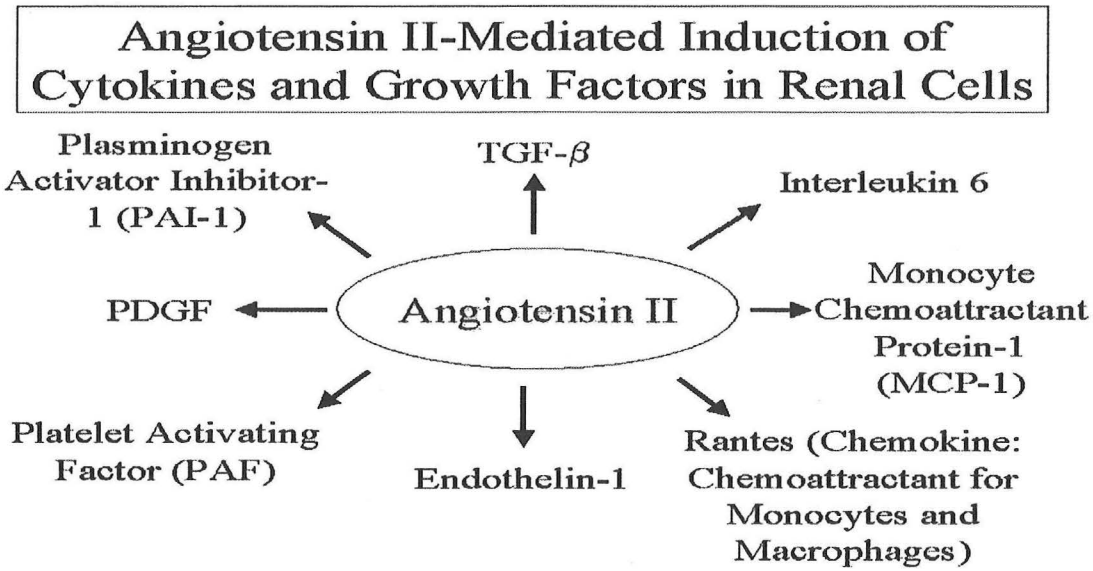
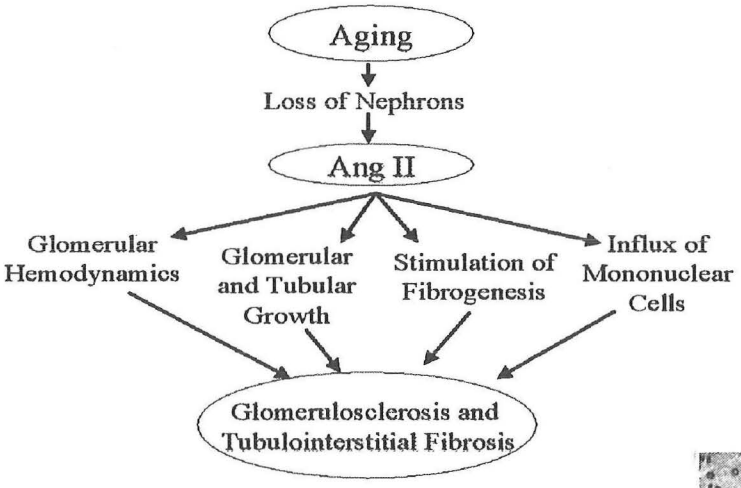


FIGURE 19



In summary, the loss of nephrons in aging seem to result in increased angiotensin II sensitivity, which then plays an important role in mediating the glomerular and tubulointerstitial sclerosis and fibrosis by activating a number of cellular processes.

FIGURE 20

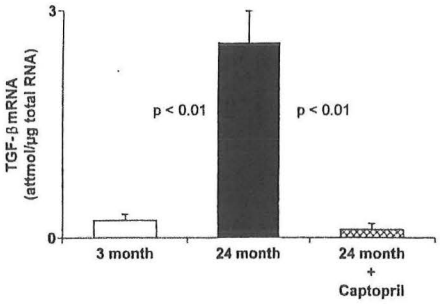


Role of TGF-β

Recent studies have also shown that renal TGF-β mRNA abundance and TGF-β immunostaining in the renal interstitium are increased as a function of aging (73,74). Furthermore, the renal protective effects of long term angiotensin II antagonism is associated with TGF-β downregulation (73).

FIGURE 21

Effect of Angiotensin II Antagonism on TGF-β_1 mRNA Expression in Aged Rats



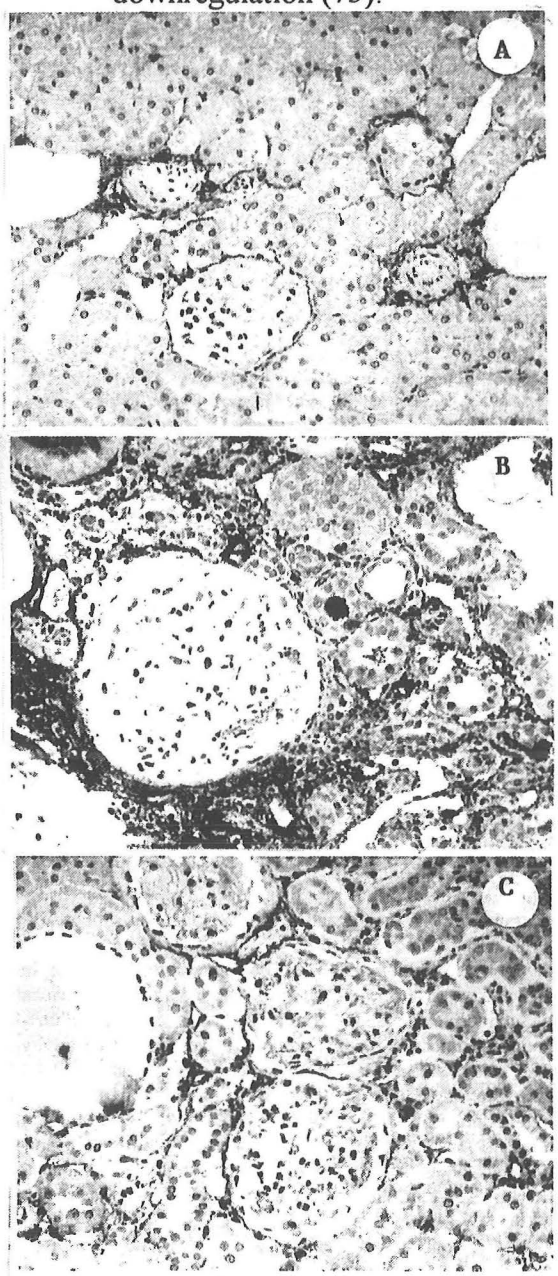
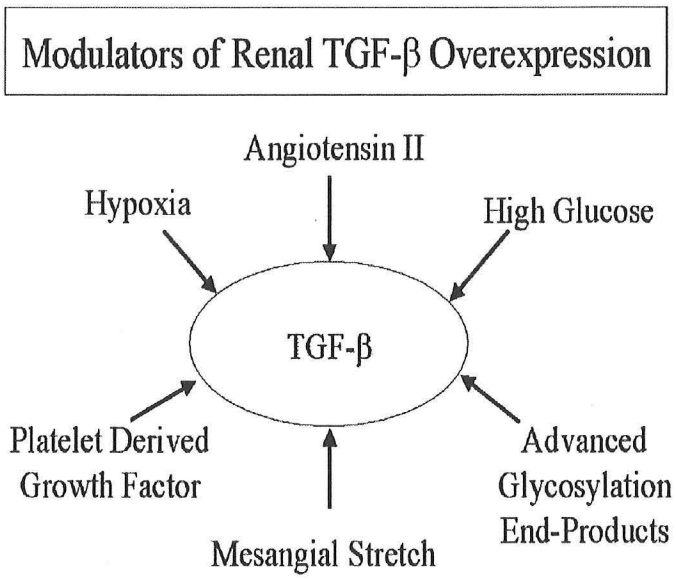


FIGURE 22. Transforming growth factor-β (TGF-β) immunostaining in 3- and 24-mo-old rats. (A) Negative control with primay antibody and normal pattern of immunostaining in 3-mo-old rats (streptavidin-biotin-alkaline phosphatase, x200). Note the characteristic perivascular and periglomerular staining and the mild interstitial deposits of TGF-β. (B) Interstitial, tubular, and periglomerular immunostaining in 24-mo-old rats (streptavidin-biotin-alkaline phosphatase. Note the presence (x200) of strong interstitial and periglomerular staining for TGF-β in areas with severe fibrosis. (C) TGF-β immunostaining in the renal cortex of a captopril-treated 24-mo-old rat (x200). Note the similar appearance to control rats. (73)

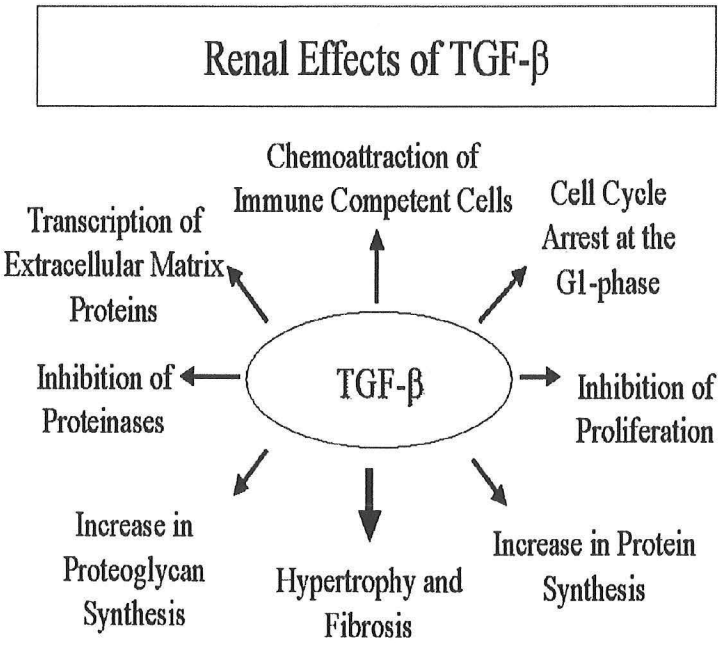
Potential pathophysiological processes that may lead to increased renal expression of TGF-β in the aged kidney include increased angiotensin II activity, abnormal glucose metabolism, increased levels of advanced glycosylation end products (AGEs), hypoxia and/or oxidative stress.

FIGURE 23



TGF-β stimulates gene transcription and the production of collagen III, IV, and I. TGF-β also stimulates the production of fibronectin, tenascin, osteonectin, osteopontin, thrombospondin, and matrix glycosaminoglycans. In addition, TGF-β inhibits collagenase and transcription and stimulates the synthesis of metalloproteinase inhibitors. The net result is the accumulation of extracellular matrix proteins resulting in glomerulosclerosis and tubulointerstitial fibrosis, as has been repeatedly shown in diabetic nephropathy and other models of progressive renal scarring (68, 69, 75, 76).

FIGURE 24



Although it is quite likely that increased expression of TGF-β mediates, in part, the age-related sclerosis, it should be noted that the definitive proof is still lacking. Approaches which have been used in experimental models of glomerulo-nephritis, including using neutralizing antibodies to TGF-β, decorin which antagonizes the action of TGF-β, or antisense oligonucleotides which specifically inhibit TGF-β expression, may establish a more definite role for TGF-β in mediating the age-related renal dysfunction.

Role of Nitric Oxide

There is increasing evidence that nitric oxide plays an important role in the regulation of renal function and glomerulosclerosis and tubulointerstitial fibrosis in the aging kidney.

Urinary excretion of the stable NO oxidation products nitrate + nitrite, is decreased with age (77, 78). In addition, there is also decreased expression of endothelial nitric oxide synthase (eNOS) in the peritubular capillaries of aged rats (79).

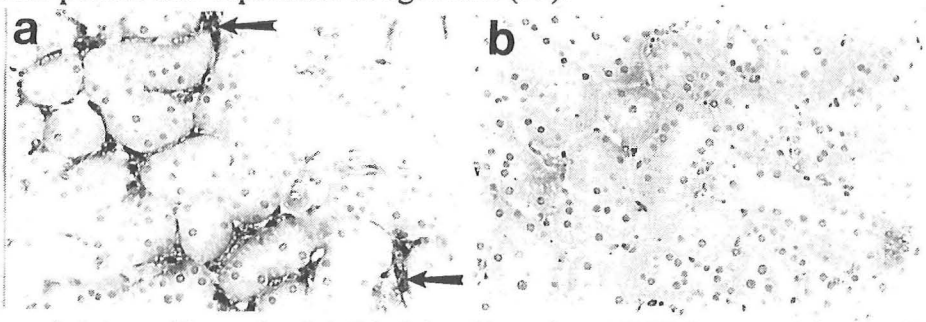
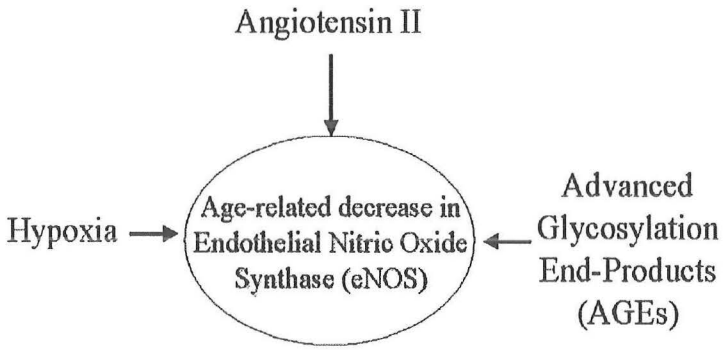


FIGURE 25. Peritubular capillary and endothelial nitric oxide synthase (eNOS) immunostaining are altered in the aging rat. Photomicrographs show differential eNOS immunostaining and peritubular capillary structure in young and aging rats. (a) Immunostaining for eNOS by the peritubular capillaries in the young rats. Note the relative absence of eNOS staining in the peritubular capillaries of the aging rats (b).

The causes for the age-related decrease in endothelial nitric oxide synthase are not known, but potential causes include increased angiotensin II activity, increased levels of advanced glycosylation end products, the hypoxia and/or oxidative stress and perhaps increased dietary protein intake (80-84). Treatment of aged rats with angiotensin II antagonists and/or dietary protein restriction results in significant increases and normalization of urinary NO excretion (77).

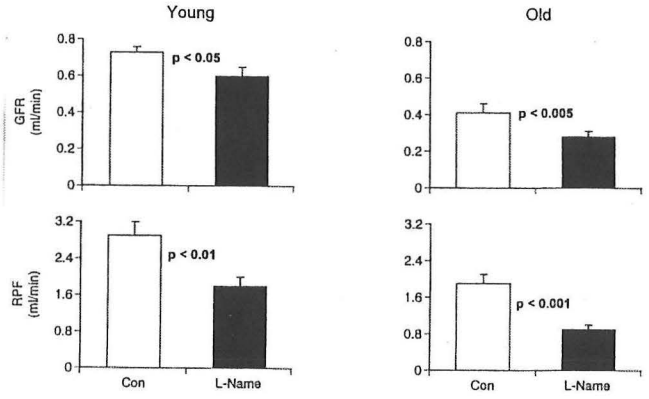
FIGURE 26



Inhibition of nitric oxide activity with L-NAME, an inhibitor of NO synthesis, causes significant decreases in RBF and GFR and an increase in RVR in both the young and aged rats. The hemodynamic changes are however more marked in the aged rat, which indicates that the aged rats are more dependent on NO for maintenance of renal perfusion. In addition, in the absence of NO it also indicates increased renal vasoconstrictive effect of unopposed angiotensin II action in the kidney (85-89).

FIGURE 27

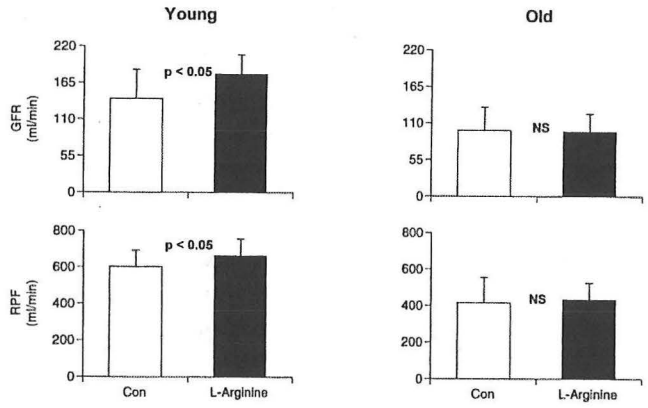
Role of Nitric Oxide in Regulation of Renal Function in Aged Rats



Infusion of the NO substrate L-arginine has similar vaso-dilatory effects in both young and aged rats. On the other hand, in hypertensive humans the renal response to L-arginine infusion is abolished in the aged subjects. While L-arginine causes significant increases in RBF and GFR in young subjects, the aged subjects show no response to L-arginine infusion (90).

FIGURE 28

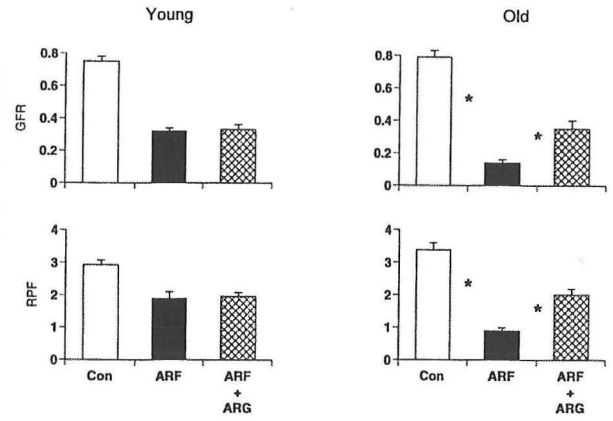
Aging Abolishes the Renal Response to L-Arginine Infusion



Nitric oxide also may play a role in ischemic acute renal failure in aging rats. Similar to the clinical experience with aged subjects, following renal ischemia/reperfusion (clamping of the renal arteries for 30 minutes followed by restoration of blood flow) the aged rats also develop more severe acute renal failure compared to identically treated young rats (91-92). Pretreatment of aged rats with L-arginine resulted in a significant improvement in GFR in the aged but not young rats.

FIGURE 29

Role of Nitric Oxide in Acute Renal Failure in Aged Rats



The decreased expression of endothelial nitric oxide synthase (eNOS) in peritubular capillaries may also contribute to the development of chronic tubulo-interstitial ischemia and tubulo-interstitial injury associated with aging (79). In fact, long-term dietary supplementation of aged rats with L-arginine results in significant increases in RBF and GFR and decreases in protein-uria and glomerulosclerosis(93).

FIGURE 30
Long-Term Dietary Supplementation with L-Arginine Prevents Age-Related Reduction in Renal Function

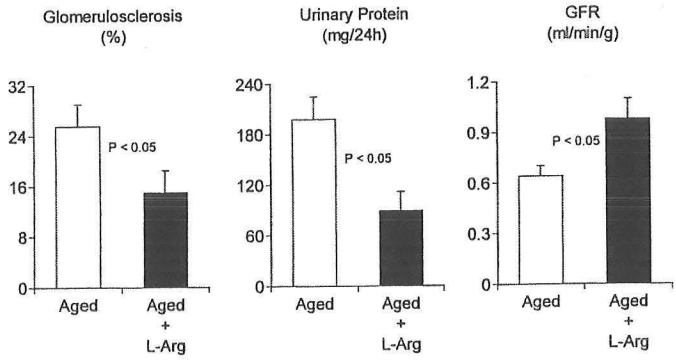


FIGURE 31
L-Arginine Reduces Kidney Collagen Accumulation In Aged Mouse

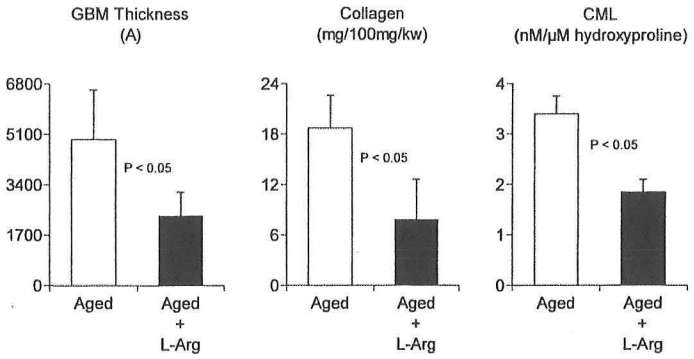
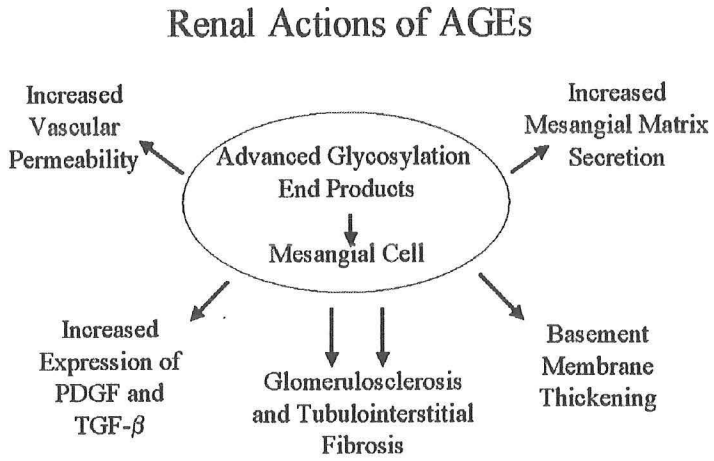


FIGURE 32



In addition, dietary L-arginine supplementation also results in significant decreases in kidney collagen and N-ε-(carboxymethyl) lysine accumulation (94).

Role of Advanced Glycosylation End Products (AGEs)

Advanced glycosylation end products (AGEs) have been shown to play an important role in the pathogenesis of diabetic renal, retinal and cardiovascular disease. For example in mesangial cells AGEs cause increased mesangial matrix secretion, basement membrane thickening, increased vascular permeability, and induction of PDGF and TGF-β, resulting in glomerulosclerosis and tubulointerstitial fibrosis (95).

Evidence for AGEs in playing an important role in diabetic renal disease is provided by the facts that: **i)** there is tissue accumulation of AGEs and increased expression of AGE receptors (RAGE); **ii)** in vivo administration of AGE-modified proteins to normal animals, including albumin and LDL, reproduces the typical diabetic lesions and proteinuria; **iii)** treatment with Aminoguanidine, an inhibitor of AGE formation, prevents the structural and functional deficits in AGE-treated normal animals and in diabetic animals.

There is also ample evidence that AGEs accumulate in several tissues including in the kidney of aged humans and animals (95-100). Increased expression of AGEs and the receptor for AGEs (RAGE) have been convincingly demonstrated both by biochemical assays and by immunohistochemistry.

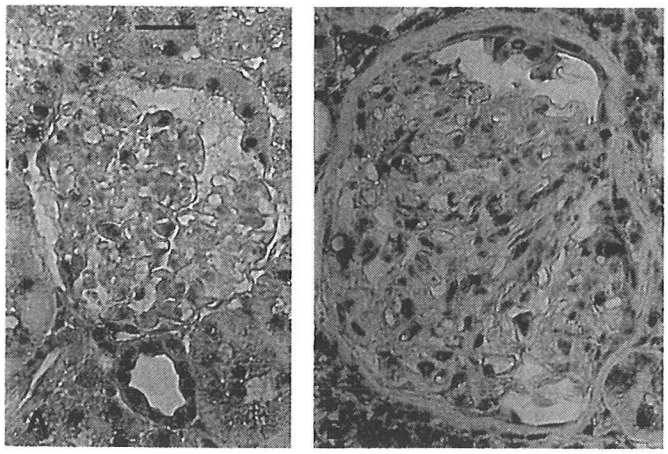


FIGURE 33. Immunohistochemical detection of advanced glycosylation end products (AGEs) in glomerular sections is shown from young rats (left), and from old untreated rats (right)(111).

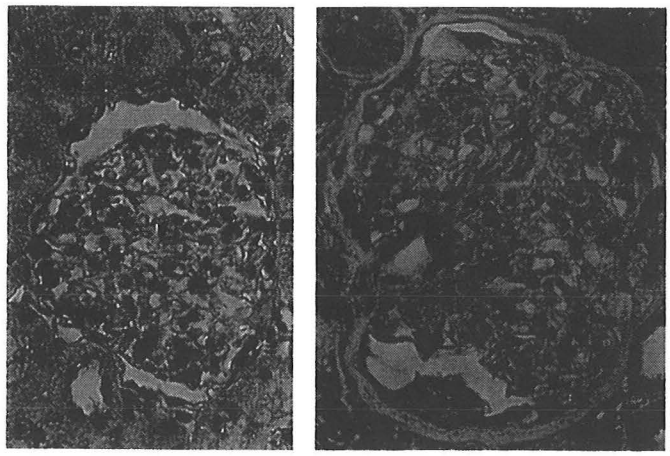
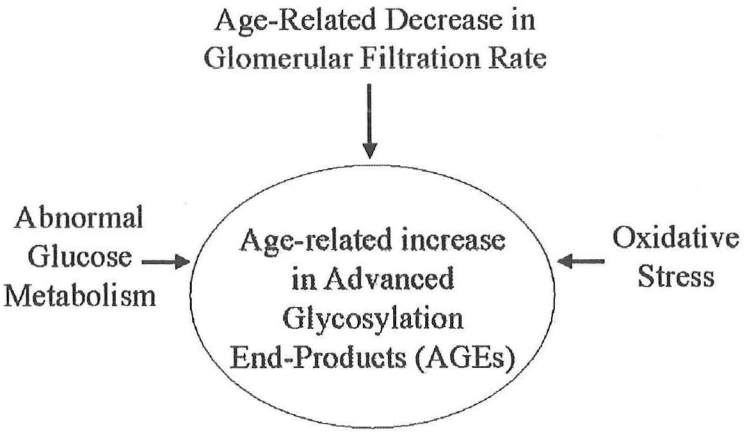


FIGURE 34. Immunohistochemical detection of RAGE in glomerular sections in young rats (left) and old untreated rats (right). (111).

The potential causes for the age-related increases in AGEs and RAGE include: a) age-related decrease in GFR, which results in decreased renal clearance and increased plasma levels; b) age-related increase in oxidative stress, which causes oxidative modification of glycated proteins and results in the accumulation of N-ε-(carboxymethyl)lysine; c) age-related insulin resistance resulting in abnormal glucose metabolism and glycation of proteins.

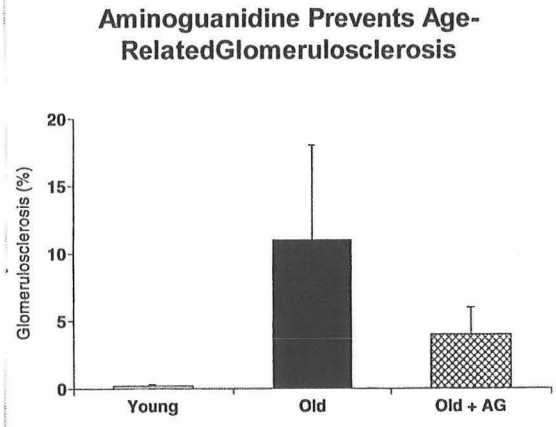
FIGURE 35



Some but not all studies have shown that caloric restriction, which has been shown to increase the life span and lessen the renal lesions in aged animals, may also attenuate the accumulation of AGEs, including N-ε-(Carboxy-methyl) lysine (CML) and pentosidine, major products of oxidative modification of glycated proteins (101, 102).

A role for AGEs in playing a significant role in age-related renal and cardiovascular disease has been recently demonstrated in aged rats treated long-term with Aminoguanidine. Treatment with Aminoguanidine markedly diminished a) the glomerulosclerosis and proteinuria (103) and b) the age-related arterial stiffening and cardiac hypertrophy (104).

FIGURE 36



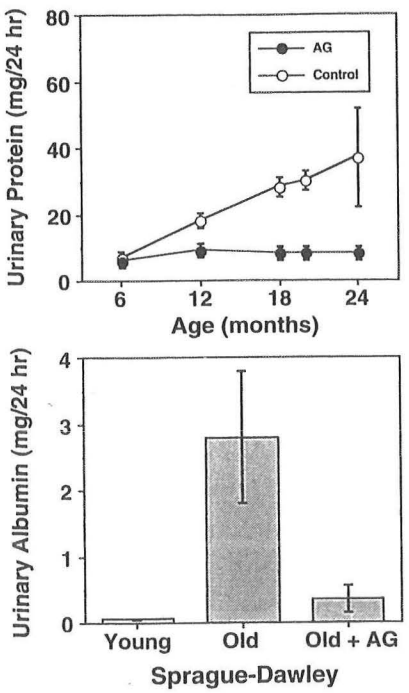


FIGURE 37. Age-related urinary protein (top) and albumin loss (bottom) in the rat is suppressed by AG. Total urinary protein concentration was determined in 24-hr urine colleced every 4 mo over an 18-mo period from S-D (top) rats treated with AG (0.1% in drinking water) (●) or untreated, age-matched controls (○). Albuminuria was determined at baseline and at the end of the study in S-D (bottom) rats. Data are expressed as means ± SEM. Comparisons (old vs. young; old vs. old + AG) of all experimental groups of both strains were significant at $P<0.05$ (n=5-7 rats per group)(103).

Role of Increased Oxidative Stress

In recent years there has been increasing evidence for age-related increase in free radical production and/or antioxidant enzyme deficiency that leads to lipid peroxidation and oxidative stress, resulting in tissue injury (105-108).

A recent study found that the levels of oxidized proteins are increased in the skeletal muscle of aged rats, which was reflected by increased levels of oxidized amino acids in the urine as well. Antioxidant therapy and/or exercise resulted in a marked decrease in the levels of oxidized amino acids (109).

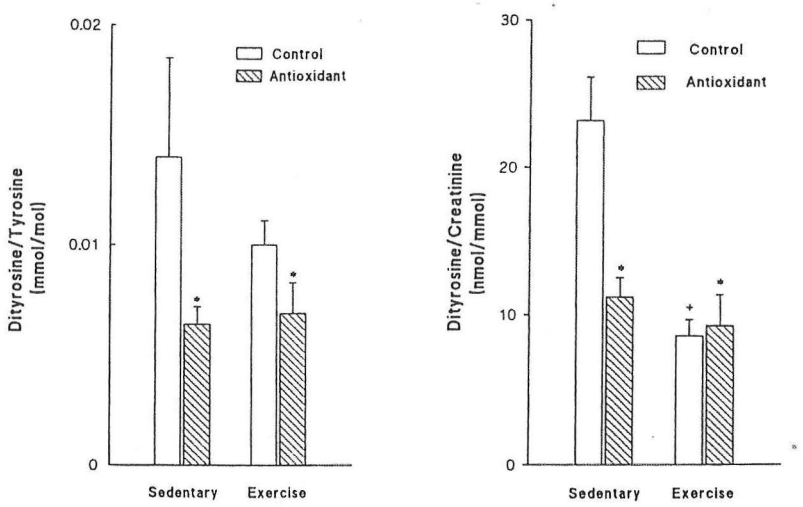
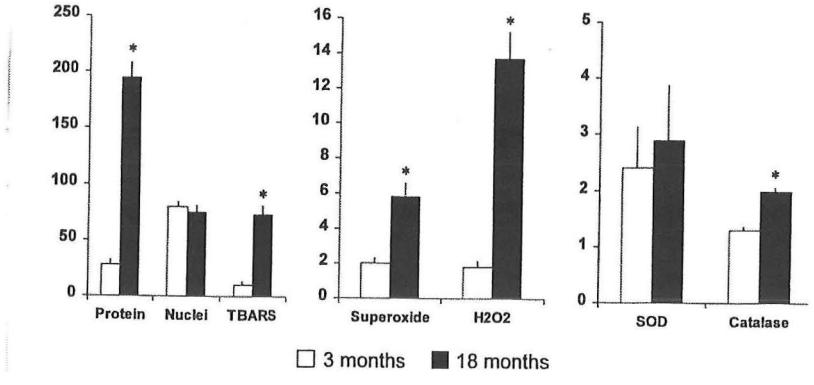


FIGURE 38. Protein-bound 0,0'-dityrosine (left) in skeletal muscle of rats. At 5 mo of age female animals were continued on control diet or fed control diet supplemented with antioxidants (ascorbic acid, α-tocopherol, butylated hydroxytoluene, and β-carotene). One-half the animals in each group had access to a running wheel for exercise. Rats were killed at 24 mo of age, and levels of oxidized amino acids in acidy hydrolysates of skeletal muscle were quantified by isotope dilution gas chromatogrphy-mass spectrometry (GC-MS). Tissue contents of 0,0'-dityrosine are normalized to content of precursor amino acids tyrosine. Results represent means ± SE (n=6/group) *P<0.05 compared with animals fed control diet. Levels of 0,0'-dityrosine (right) in urine of rats. When animals were 24 mo of age urine was collected during an overnight fast. Levels of oxidized amino acids in urine were quantified by isotope dilution GC-MS. O,o'-ityrosine are normalized to creatinine content of urine. Results represent means ± SE (n =6/group). *P<0.05 antioxidant vs. control group. +P<0.005 compared with sedentary control animals. (Ref. 109)

The activities of antioxidant enzymes are decreased in the kidneys of aged rats (105). At the same time, the levels of reactive oxygen species and thiobarbituric acid reactive substance (TBARS), an indicator of lipid oxidative damage, are increased in the aging kidney (110).

In Aged Rats There Is an Imbalance Between Reactive Oxygen Generation and Antioxidant Enzyme Activity

FIGURE 39



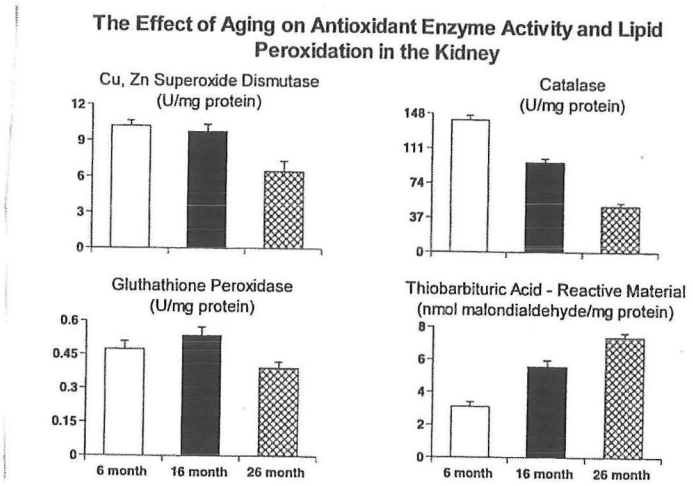


FIGURE 40

A recent study in aged rats also found evidence for increased oxidative stress and lipid peroxidation in the kidney, including increased formation of isoprostanes, increases in AGEs, the receptor for AGEs (RAGE) and induction of heme oxygenase (111). Treatment of these rats with a high-vitamin E diet caused attenuation of these changes and resulted in significant improvements in renal blood flow and glomerular filtration rate and a decrease in glomerulosclerosis.

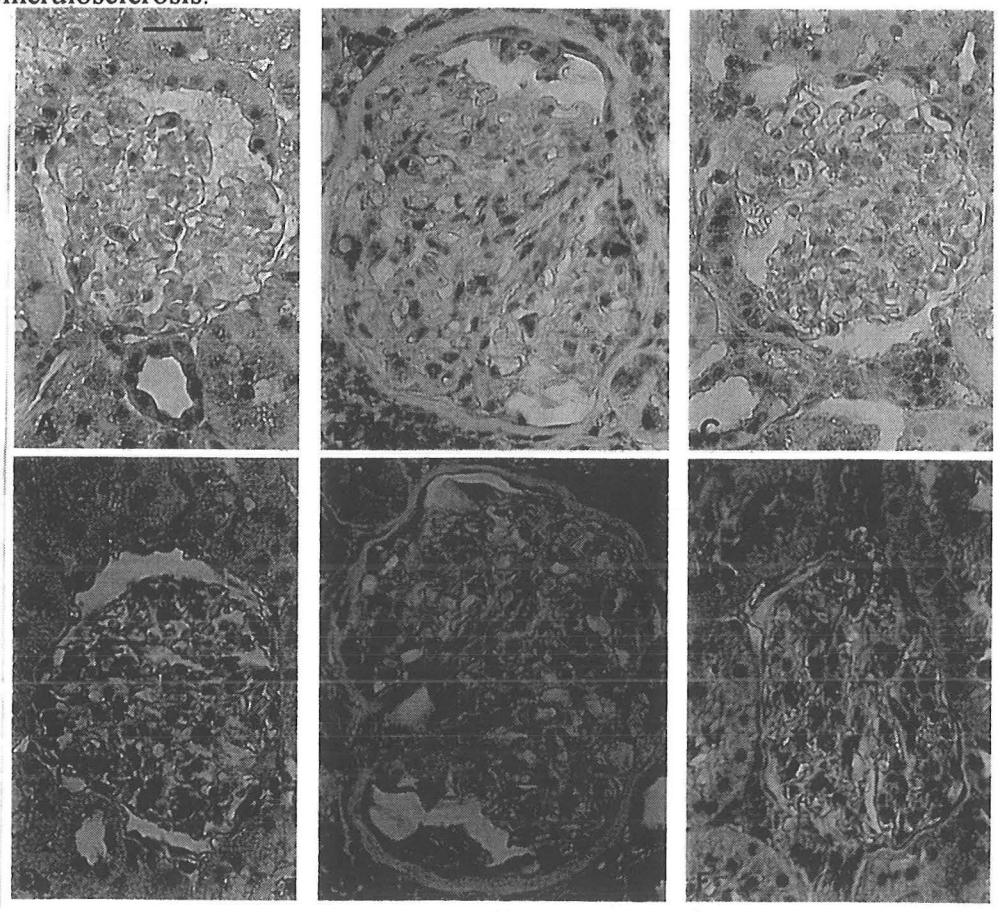


FIGURE 41

AGE

FIGURE 42

RAGE

YOUNG **AGED** **AGED + VITAMIN E**

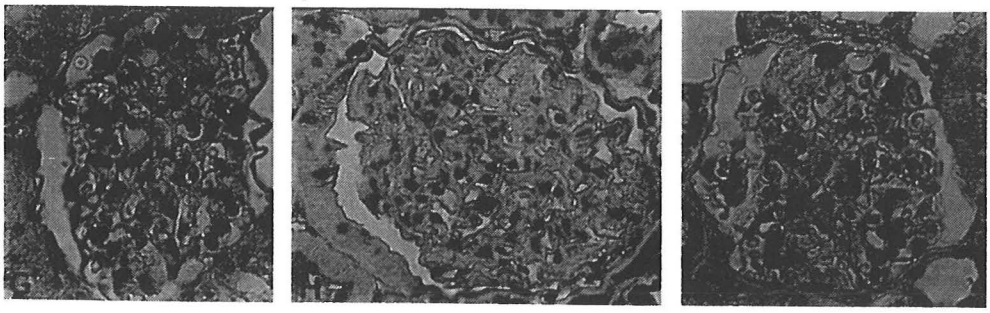


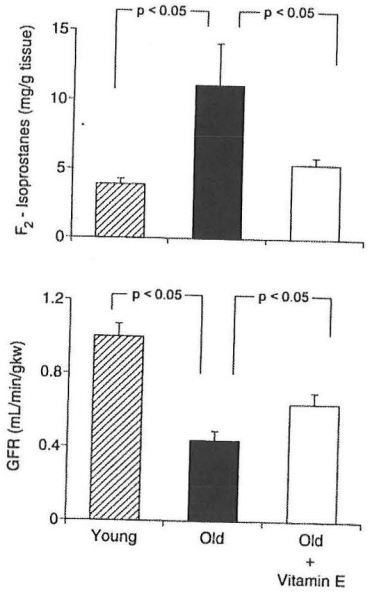
FIGURE 43

HEME OXYGENASE

YOUNG **AGED** **AGED + VITAMIN E**

FIGURE 41, 42, 43. Immunohistochemical detection of advanced glycosylation end products (AGEs), AGE receptor (RAGE), and heme oxygenase in kidneys and vascular sections from young rats, aged 3-4 mo, and from old rats, aged 22 mo, given either a control diet or a high-vitamin E diet for 9 mo. Figure 41, 42 and 43 are representative glomerular sections. Sections from young rats are in the first panel of each figure. Sections from old untreated rats are in the second panel in each figure. Last panels in each figure are sections from old rats that received high-vitain E deit. Immunohistochemial detection of AGEs in glomerular sections is shown in Figure 41. Immunohistochemical detection of RAGE in glomerular sections is shown in Fgure 42. Immunohistochemical detection of hemeoxygenase in glomerular sections Is shown in figure 43. Bar, 18 μm. (111).

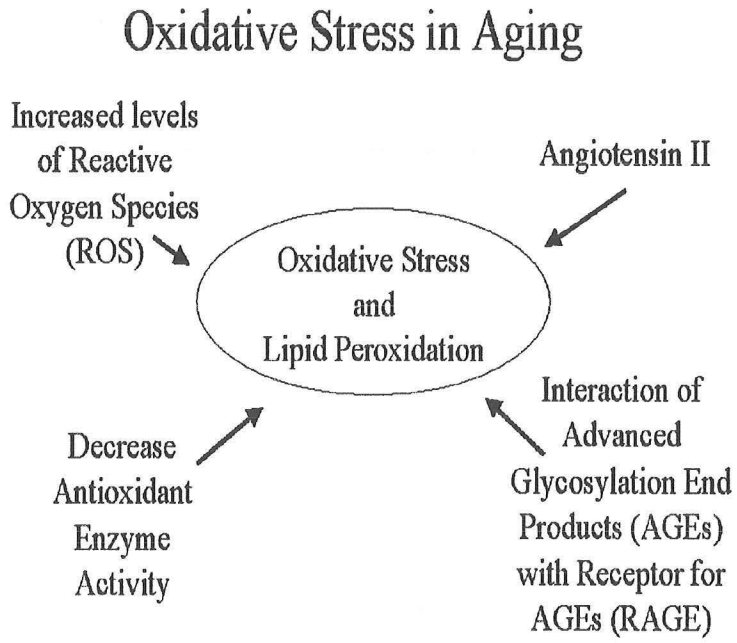
FIGURE 44



Recent studies have shown that angiotensin II stimulates superoxide production by activating the membrane-bound NADH/NADPH oxidase and that angiotensin converting enzyme inhibitors increase the activity of antioxidant enzymes (112,113).

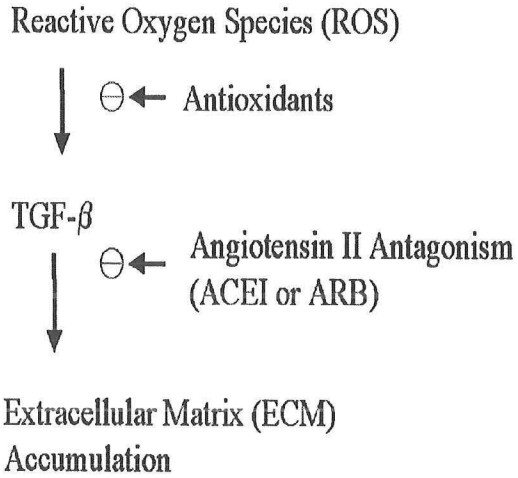
In addition increased levels of reactive oxygen species, decrease in antioxidant activity, and increased activity of angiotensin II, an additional mechanism for the increase in oxidative stress and lipid peroxidation in aging is the increased interactions between advanced glycosylation end products and their receptor (RAGE).

FIGURE 45



Studies in progress indicate that in mesangial cells reactive oxygen species result in induction of TGF-β which in return causes an increase in extracellular matrix synthesis. Treatment of isolated mesangial cells or aged rats with antioxidants and/or ACE inhibitors block these processes (Diego Rodriguez Puyol et al, unpublished data).

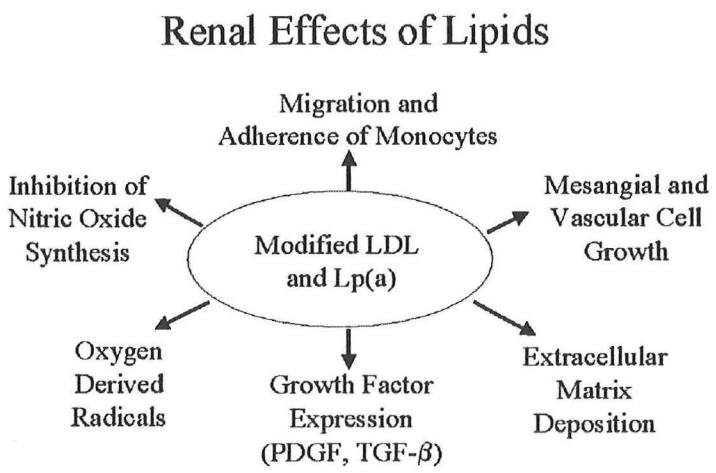
FIGURE 46



Role of Cholesterol

Recent studies suggest that cholesterol plays an important role in the progression of glomerulosclerosis and proteinuria, in a number of disease states, including in diabetes (114-117). There is age-related increased accumulation of cholesterol in several tissues, including in the kidney (118-125). In addition, because of age-related increases in oxidative stress and advanced glycosylation end products, there is also the possibility of increased levels of modified LDL and Lp(a). These lipid products have been shown to have multiple effects in the kidney and could play an important role in the pathogenesis of age-related renal disease (126, 127, 128).

FIGURE 47

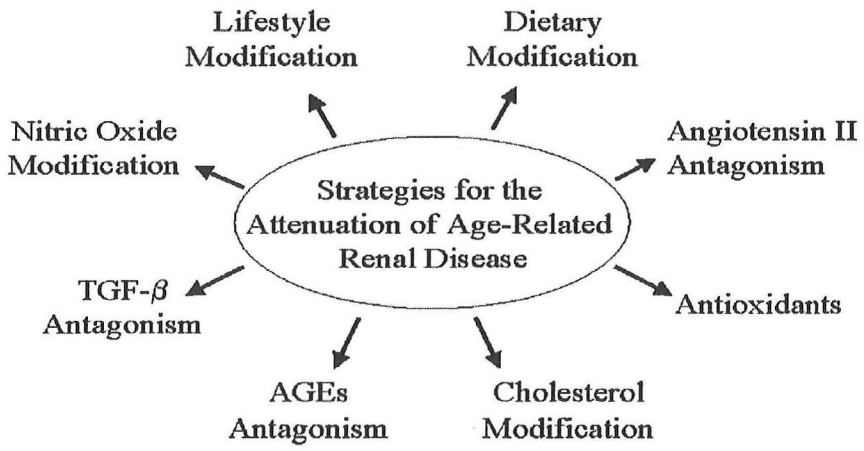


Unfortunately, there have been no studies showing that cholesterol mediates the age-related glomerulosclerosis and tubulointerstitial fibrosis. Two recent studies in subjects with type II diabetes, however, are definitely encouraging, as long-term treatment with HMG-CoA reductase inhibitors resulted in decrease in proteinuria and partial preservation of glomerular filtration rate (129, 130). Obviously, similar trials also need to be considered in aged individuals.

SUMMARY

In summary I hope that the material covered in this Medicine Grand Rounds has provided ample convincing evidence that there are multiple factors which play a role in the pathogenesis of the age-related renal disease. Long-term interventions to modify these processes can hopefully prevent or at least significantly attenuate the age-related renal disease.

FIGURE 48



ACKNOWLEGEMENTS

I would like to dedicate this Medicine Grand Rounds to the beloved memory of Dr. Seymour Eisenberg, who upon my arrival in Dallas 15 years ago encouraged my interest in the biology and pathophysiology of the aging kidney. He was and will continue to be a source of inspiration for me.

I would like to express my gratitude to Dr. Robert Schrier, Dr. Donald Seldin and Dr. Barry Brenner who each gave me and my colleagues a chance to write about the Aging Kidney in their nephrology textbooks. In this regard, I would also like to thank my colleagues Dr. Devasmita Dev and Dr. Biff Palmer from University of Texas Southwestern Medical Center and Dr. Victor Sorribas from University of Zaragoza, Spain for sharing my interest in the aging kidney.

Finally, I thank a) my secretary Teresa Autrey, b) Sharon Balthrop and the Dallas VA Medical Media Department and c) Shirley Campbell and the Dallas VA Library Service for helping me to put together this review.

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