Kidney function declines with age in the majority of the population. Although very few older people progress to end stage, the consequences of doing so are burdensome for the patient and very expensive for the society. Although some of the observed decline is likely due to changes in the vasculature, much is associated with the development of age-associated glomerulosclerosis. This article will review the well-established structural and functional changes in the glomerulus with age. The role of calorie restriction in modifying age-related pathology will be discussed. The importance of the podocyte as a critical cell in the aging process is considered using animal models and human biopsy material. Newer data on changes in gene expression driven by nuclear factor kappa beta (NFκB) and possible changes in biology in the glomerulus are discussed. The relationship between pathways involved in aging and the decline in kidney function is reviewed. There is speculation on the significance of these changes in relation to normal and pathological aging.

Key Words: Aging—Glomerulus—Podocyte—Calorie restriction.

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END-STAGE kidney disease (ESKD) is a disease of the aging population (Figure 1). The mean age at the start of renal replacement therapy is 62.3 years for men and 63.4 years for women (1). Peak incident counts of treated ESKD occur in the 70–79 age group at more than 15,000 patients per year. The incident rate of treated ESKD in the 70–79 year old age group is 1,673 per million population (1) and peaks at 80–85 years of age at 1,931 per million population. Glomerulosclerosis is the underlying cause of 90% of ESKD (1,2). Glomerulosclerosis is associated with aging itself and with diabetes, hypertension, and other glomerular diseases including focal segmental glomerulosclerosis and inflammatory glomerulonephropathies (2). Glomerulosclerosis identified in autopsy studies is present in more than 70% of people aged 40 years and older, with increasing prevalence and percentage of glomeruli involved by glomerulosclerosis with age (3). Renal function declines with age in association with well-described changes in structure and function (4–6). There is a strong correlation between age and worse outcome for most glomerular diseases (2). Diseases such as diabetes and hypertension are themselves common in older age and are known to accelerate glomerulosclerosis. However, this does not seem to be sufficient to account for the rates of ESKD or the increase in susceptibility to injury that occurs with age. Understanding how aging affects the glomerulus will help us to recognize who is at risk for kidney damage and how to prevent progression to ESKD in the older population.

Previous studies on age-related kidney injury are restricted to classical histological and functional analysis in humans and in rodents, with a focus on the affect of diet on these changes in rodents (3–12). They include marked widening of the glomerular basement membrane (GBM) with age, expansion of the mesangial compartment, enlargement of the glomerulus, development of glomerulosclerosis in an increasing proportion of glomeruli with age, reduction in glomerular filtration rate, reduction in capacity of the kidney to concentrate urine, and worsening outcome following glomerular injury.

The glomerulus is the filtration structure of the kidney. The vascular side is lined by fenestrated endothelial cells. These are separated from the urinary space by the basement membrane. The basement membrane is lined by
epithelial cells, known as podocytes. The whole structure is supported by the mesangial cells and their mesangial matrix. The entire glomerulus is surrounded by Bowman’s capsule, lined by parietal epithelial cells. This whole structure is responsible for filtering the circulating blood and creating urine for excretion. The key cell involved in glomerulosclerosis is the podocyte, a highly differentiated neuron-like epithelial cell with limited capacity for cell division and replacement (13–18). Podocytes function to support and maintain the GBM filtration mechanism (17). Genetic diseases that manifest a glomerulosclerotic phenotype primarily include mutations in proteins expressed by the podocyte, thereby providing support for a link between podocyte dysfunction and glomerulosclerosis (19–23). Experimental models suggest that podocyte injury and loss from the glomerulus may be a key component of the process driving glomerulosclerosis (24–27). A transgenic model where podocytes express the human diphtheria toxin receptor has been developed. It is possible to duplicate glomerulosclerosis by titrating the dose of diphtheria toxin to knock out varying percentages of podocytes (27). Loss of podocyte markers from glomeruli is also associated with glomerulosclerosis in human biopsy samples from patients with focal segmental glomerulosclerosis (28,29). Several investigators have shown increased appearance of podocytes and podocyte constituents in urine in association with glomerulosclerosis and more rapid deterioration of renal function in focal segmental glomerulosclerosis (30–32). Together, these data support the idea that the podocyte is a key component for the development of glomerulosclerosis and that a failure of podocytes to cover the available GBM filtration surface area results in denuded areas of GBM, which in turn triggers matrix accumulation and glomerulosclerosis. A key question to be addressed is whether the acceleration of ESKD prevalence in older age could somehow also be related to podocyte injury and loss. Floege and colleagues (33) have provided data to support the concept that the glomerulosclerosis of aging is “a podocyte disease” although the mechanism by which this might occur was not elucidated. The Fischer 344 rat is a model of aging developed by the National Institute on Aging for the study of aging processes. This rat does not develop diabetes or hypertension with age but does develop glomerulosclerosis with age when fed on an ad-lib diet (34).

Using Fischer 344 rats that are fed different diets, it is possible to look at how high-calorie intake might interact with age-associated glomerulosclerosis (Figure 2). It has been known since the early 1990s that calorie restriction not only results in extension of life span, but also moderates the pathologies of aging (34). Cynthia Kenyon, working in Caenorhabditis elegans, showed that the increase in longevity associated with calorie restriction occurs through interference in the insulin signaling pathway, specifically insulin-like growth factor 1 (IGF-1) (35). Since then similar data have become available for drosophila and mice (36–38). Cai and colleagues (39) showed in mice that restricting advanced glycation end products intake was as important as restricting calories in preventing renal pathology with age. Early work on Fischer 344 rats had shown that calorie restriction prevents the development of age-associated glomerulosclerosis (34). Calorie-restricted Fischer rats live 6–8 months longer than their ad-lib-fed littermates and do not develop renal pathologies even at the end of their extended lives.

With aging, the glomerulus undergoes significant enlargement with robust mesangial expansion (Figure 2).
There are appropriate increases in mesangial and endothelial cell numbers, such that the ratio between glomerular volume and cell number stays constant. It has been shown that there is a relative rather than an absolute depletion of podocytes that occurs with age (40). Podocytes undergo hypertrophy rather than hyperplasia in association with glomerular enlargement. However, as hypertrophy proceeds, it is associated with changed podocyte biology and eventually failed further hypertrophy such that there is relative podocyte depletion and associated glomerulosclerosis. Calorie restriction prevents these events from occurring (Table 1). Morphometric data show that in ad-lib-fed animals, glomerular volume increases threefold by 24 months, whereas podocytes do not increase in number (40). Through hypertrophy, podocyte mass is only able to increase 2.6-fold. In calorie-restricted animals, podocyte mass increases 1.3-fold, whereas glomerular volume increases 1.5-fold (Table 1).

Table 1. Fold-Changes in Ad-lib-Fed and Calorie-Restricted Rats in Relation to Baseline

|                | Glom Vol | Total Cells | Podocyte # | Podocyte Size |
|----------------|----------|-------------|------------|---------------|
| 2 mo           | 1        | 1           | 1          | 1             |
| 24-mo ad-lib   | 3.1      | 2.6         | 1.2        | 2.2           |
| 24-mo CalRes   | 1.5      | 1.0         | 1.1        | 1.2           |

*Notes:* The parameters listed are as follows: Glom Vol = glomerular volume; Total Cells = total glomerular cell number; Podocyte # = podocyte number per glomerulus; and Podocyte size. The numerical data provided are the fold-increase above the common 2-month (2 mo) time point measured in the ad-lib-fed and calorie-restricted (CalRes) rat groups at 24 months (24 mo) of age.

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Accompanying these morphometric changes in Fischer rats, there are also changes in glomerular gene expression with age. DNA expression profiling using microarray analysis of isolated glomerular RNA preparations shows changes in both ad-lib-fed and calorie-restricted rats during aging. To identify genes of potential importance to the glomerulosclerotic process and to minimize the age affect, the differences in gene expression between ad-lib-fed and calorie-restricted rats were examined throughout life (40). There were 497 out of approximately 30,000 probe sets (1.6%) that were significantly different with aging. Of these, 302 probe sets coded for known genes. Several podocyte marker molecules were present in the selected probe sets. The most striking result was that in each case, the difference (ad-lib minus calorie-restricted) was negative at 24 months. The probe sets identified as significant included the podocyte transcription factor WT1, mutations of which cause glomerulosclerosis in man (41); another podocyte transcription factor Pod1 (42); nephrin, the key podocyte slit diaphragm protein, which is mutated in congenital nephrotic syndrome (43); the podocyte apical membrane protein tyrosine phosphatase PTPro-GLEPP1 (44); and alpha 5 type IV collagen, mutations of which are responsible for abnormalities of GBM in Alport’s syndrome (45).

By 24 months, ad-lib-fed rat glomeruli had significantly less mRNA per total glomerular mRNA for these podocyte molecules than did the calorie-restricted rats of the same age. Quantitative real-time PCR for nephrin confirmed the finding that podocyte mRNAs were relatively decreased at the 24-month time point in ad-lib-fed rats. At 24 months, the ad-lib-fed rat’s glomeruli contained significantly less mRNA for nephrin (59%) than did calorie-restricted rats (40). This was also manifested at the protein level by measuring the nephrin protein in glomerular protein extracts from the ad-lib-fed rats. There was a statistically significant 47% decrease in nephrin protein concentration relative to total glomerular protein at 24 months in ad-lib-fed rats compared with the 17-month time point (p < .05). This result is consistent with the morphometric and glomerular mRNA data presented earlier. This would support the idea that podocyte biology changes in major ways with aging, including the loss of markers that characterize the podocyte phenotype.

Not all the gene expression changes represent decline or loss of podocyte molecules. Floege and colleagues (33) previously demonstrated that desmin, an intermediate filament, was increased in podocytes in association with aging in a rat model. Desmin is one of the genes that increased on the DNA microarray data and was significantly higher in ad-lib-fed than in calorie-restricted glomeruli. Glomerular

Figure 2. Aging glomeruli. Photomicrographs of glomeruli at different ages of ad-lib-fed and calorie-restricted rats developed with peroxidase GLEPP1 as a podocyte marker and counterstained with Periodic acid-Schiff and hematoxylin. The panels are all taken at the same magnification. Left: 2-month glomerulus. Center: 24-month calorie-restricted glomerulus. Right: an example of a partially sclerosed glomerulus at 24 months of age in the ad-lib-fed group. Note the increase in glomerular size and the expanded mesangial compartment in the ad-lib-fed older rats compared with calorie-restricted rats. The size bar in each photomicrograph is 50 µm long.
desmin mRNA is increased significantly in ad-lib-fed rats by 17 months and 24 months as assessed by real-time PCR measurements. Desmin protein is also increased at these time points as assessed by protein quantitation in glomerular extracts. Desmin mRNA levels remain low in calorie-restricted rats (40). This result suggests that even by 17 months in ad-lib-fed rats, before proteinuria is present and glomerulosclerosis has appeared, the biology of the podocyte has changed in a significant way.

Although intact structure and function of the podocyte appears to be critical to glomerular health, changes in biology with age are not limited to podocytes. Glomerular affymetrix DNA expression profiling demonstrated significant increases in ceruloplasmin (Cp) expression in the parietal epithelial cells in ad-lib-fed rats (46). Cp is a copper-containing ferroxidase that functions as an antioxidant in part by oxidizing toxic ferrous iron to nontoxic ferric iron (47–49). High-calorie intake increases free radical production and oxidation of key biomolecules. Fischer 344 rats maintained on an ad-lib diet develop oxidant injury and age-associated glomerulosclerosis by 24 months (50,51). Calorie restriction prevents both oxidant injury and glomerulosclerosis. In ad-lib-fed rats, Cp mRNA expression increased by sixfold \( (p < .01) \) and protein expression increased by fivefold \( (p = .01) \) between 2 and 24 months of age. In calorie-restricted rats, Cp mRNA expression increased by threefold \( (p < .01) \) and protein expression increased 1.6-fold (NS) between 2 and 24 months of age (46). Both the cell-associated alternately spliced variant and secreted variant were expressed. This expression was localized to the parietal epithelial cells lining the inner aspect of Bowman’s capsule of the glomerulus. Cp was also present in urine, particularly of old ad-lib-fed rats with high tissue Cp expression (46). Thus, Cp expression at this site may be part of the repertoire of the glomerular parietal epithelial cell to protect the glomerular podocytes and downstream nephron from toxic effects of filtered molecules including ferrous iron and is driven by the aging process in the glomerulus.

The gene expression changes are highlighted earlier, and the probable resulting changes in biology are all increased by ad-lib feeding and reduced by calorie restriction. The affymetrix gene expression profiling, however, identified another group of genes. In this group, the level of expression changed linearly (either up or down) throughout the period of adult aging from 2 to 24 months and was not different between calorie-restricted and ad-lib-fed animals, suggesting that they were a manifestation of aging rather than pathology. DNA profiling identified 163 genes in this group. These genes represent proteins that are characteristic of each of the different cell types in the glomerulus (52). This group included vascular cell adhesion molecule and intercellular adhesion molecule from the endothelial cells and MMP9 from the mesangial cells in addition to those discussed previously. Studies of the regulatory regions of these genes showed that they all had a nuclear factor kappa beta (NFkB) consensus binding site in their regulatory regions. The NFkB pathway has previously been identified as an activator of age-related transcriptional changes (53–55). In aging Fischer 344 rats, NFkB translocates to the nuclei of glomerular cells. Antibodies to NFkB can immunoprecipitate the regulatory regions of many of these genes using chromatin immunoprecipitation analysis, showing that it is actively bound to DNA at the time of these changes (Figure 3). It is not clear what is driving the increase in NFkB activity in the glomerulus, but this change alone could explain the increased susceptibility to injury and development of glomerulosclerosis.

In addition to these coordinated gene expression changes, there are changes in glomerular genes with age that are not part of the typical cellular phenotype. In a genetic screening process in aging Fischer 344 rats, 92 glomerular genes were found that appear to be silenced at 2 months but had robust expression by 24 months (52). The most striking example was prepronociceptin, a neuronal gene that has been well characterized as a modulator of pain signals in both the central and peripheral nervous system. It has also been shown to be active in the purinergic nervous system of the gut. It is not seen in the young glomerulus but appears in the podocyte with increasing age. The appearance of genes that are not part of the normal cellular phenotype would suggest that epigenetic dysregulation plays a role in the change in glomerular biology with age. Genes that might have been silenced during nephrogenesis may be turned on as the mechanisms that mark genes as silent decline. Podocytes probably do not undergo replication, so these epigenetic marks will not be renewed at cell division and may decline in efficiency with age.

There are several genetic pathways that are now known to affect the rate of aging. The first of these was the IGF-1 pathway first described by Cynthia Kenyon in 1993 as a daf-2 mutation in Caenorhabditis elegans (35). Since that initial finding, several more pathways have been added to the list, including the mTOR pathway, the sirtuin family, the autophagy pathway, and genes associated with progerias. Several of these genes, when modified in experimental animal models have a glomerular phenotype. In mice, podocyte-specific knockouts of players in the autophagy pathway have been created (56). These mice show accelerated glomerular aging with accumulations of oxidized and ubiquitinated proteins, endoplasmic reticulum stress, and proteinuria, and ultimately to late-onset glomerulosclerosis. Damaged proteins and organelles accumulated in the podocytes had a detrimental effect on cellular homeostasis, and the authors showed a decline in podocyte numbers per glomerulus compared with controls by 22 months. Declines in autophagy proteins have been demonstrated in biopsies of people with glomerular diseases (56). There is one described report of histopathology from two participants who died from progeria. The younger participant who died aged 11 years had no glomerulosclerosis, whereas
the kidney from the 20-year-old participant showed focal renal scarring with focal glomerulosclerosis and associated tubular atrophy, similar to that seen in physiological aging (57). Klotho is produced by the distal tubular cells in the kidney and overexpression is associated with increased longevity. It appears to act through the IGF-1 pathway and its role in phosphorus homeostasis is not thought to be a significant factor in its relationship to extension of life span. There is currently no data to link aging in the kidney to the sirtuin family of genes.

There is much debate within the renal community about the clinical significance of these changes. Does the change in glomerular structure and function constitute disease or normal aging? (58,59) There is very clear evidence that as kidney function or glomerular filtration rate declines, mortality and morbidity increases (60). However, only a very small fraction of these older patients will progress to ESKD or die of renal failure. This raises the issue of whether the morbidities and renal failure are parallel manifestations of a single pathological process or whether one condition is causative for the other. This debate is likely to continue until we have a greater understanding of the links between aging and the chronic diseases associated with age.

In conclusion, in addition to the well-established morphometric changes, there are important changes in gene expression and probably also in biology in the glomerulus as animals and people age. There is still much to be learned about this important aspect of aging. Links between well-known modifiers of the aging process and changes in the kidney function remain to be established. Further understanding of these processes will hopefully help to predict who is at risk from kidney failure and prevent progression to ESKD and the need for renal replacement.

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