Microalbuminuria is a recently recognized link between cardiovascular and renal damage in hypertensive diabetic and nondiabetic subjects. Many patients with high blood pressure have a renal abnormality. However, the exact relationship between increased glomerular pressure that may be derived from increases in systemic blood pressure, or the specific increases in local intraglomerular pressure without similar changes in systemic pressure and glomerular malfunction have yet to be determined.

Although the underlying mechanism responsible for albuminuria in hypertension remains speculative, recent studies suggest that factors other than changes in glomerular permeability should be considered. These studies have demonstrated that albumin excretion in healthy controls is associated with comprehensive degradation (>95%) by lysosomes in renal cells distal to the glomerular basement membrane (GBM). Previously it was assumed that virtually all proteins filtered by the kidney were excreted intact. In fact <5% of albumin is excreted intact and detectable by conventional immunochromatographic assays. The remaining >95% is degraded and undetectable by routine immunochromatographic assays. The lysosomal processing of filtered albumin before its excretion has been shown to be an extremely rapid and efficient process. It occurs within minutes and involves lysosomal uptake and exocytosis of peptide products back to the tubular lumen by renal cells distal to the GBM. There is negligible contribution of albumin fragments arising from proteolytic activity on the luminal surface of the tubular cells from extra renal sources, or from contraluminal uptake of albumin with subsequent degradation.

Similar rapid lysosomal processing has been demonstrated in vivo associated with the filtration and excretion of dextran sulfate in which the dextran sulfate is completely desulfated but not depolymerized. The ratio of intact to degraded forms of albumin and dextran sulfate has been shown previously to increase in experimental diabetic nephropathy. The increased ratio of intact to degraded albumin has also been shown in type 1 diabetic patients with diabetic nephropathy. Furthermore, aminoguanidine (an antiglycation agent) and ramipril (an ACE inhibitor) have been demonstrated to normalize the protein fragmentation/desulfation process in rats with streptozotocin-induced diabetic nephropathy.

### Key Words:
- albuminuria
- lysosomal processing
- glomerular permeability
- dextran sulfate
- transforming growth factors

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**Abstract**—Increased intraglomerular pressure is considered a major factor for increased albumin excretion in hypertension. However, other factors should also be considered because recent studies in both humans and rats have demonstrated that proteins undergoing filtration and renal passage are extensively modified by renal cell lysosomal processing; >95% of albumin is degraded to peptides that are not detected by routine immunochromatographic assays. Changes in postglomerular lysosomal processing may therefore be responsible for the increased intact albumin excreted in hypertension-related kidney disease. We hypothesize that transforming growth factor-β, which is known to decrease lysosomal activity, may be upregulated in hypertension and may play a role in increased intact albumin excretion. The aims of this study were to determine the effect that hypertension has on (1) renal cell lysosomal processing of albumin and dextran sulfate, (2) glomerular permeability, and (3) renal transforming growth factor-β expression. Spontaneously hypertensive rats and Wistar-Kyoto rats were used at 8, 16, and 24 weeks. We demonstrate that albuminuria in hypertension is linked to an inhibition of lysosomal processing as determined by (1) size exclusion chromatography analysis of urinary [14C]albumin structural integrity and (2) ion exchange analysis of urinary [3H]dextran sulfate. This inhibition gives rise to an increased proportion of radioimmunoassay detectable (intact) albumin and intact dextran sulfate independent of changes in glomerular capillary wall permeability as determined by the fractional clearance of [3H]Ficolls of various radii. These changes may be correlated with increased renal transforming growth factor-β expression. *(Hypertension. 2002;39:281-286.)*

**Correspondence** to Wayne D. Comper, Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia 3800. E-mail wayne.comper@med.monash.edu.au

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These results may be linked to changes in transforming growth factor-β (TGF-β) expression, which is upregulated in diabetes and other kidney disease states and may be upregulated in hypertension as a result of increased stretching forces brought about by the hypertensive state. Recent studies have shown that lysosomal activity may be affected by increased TGF-β levels, and its role in albuminuria induction may be further supported by the fact that ACE inhibitors and decreasing albuminuria are correlated with decreased TGF-β levels, which leads to the hypothesis to be tested in this study.

Normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) male rats were used to study the nature of urinary albumin excretion associated with the hypertensive state. Changes in lysosomal processing were determined by size exclusion chromatography analysis of urinary [14C]albumin administered by osmotic pump. Ion exchange chromatographic analysis of urinary [3H]dextran sulfate administered intravenously was used to detect changes in lysosomal sulfatase activity. These changes were correlated with changes in renal TGF-β1 expression as determined by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) for TGF-β1 mRNA and further confirmed with the upregulation of the TGF-β-inducible gene-h3 mRNA, also by RT-PCR. Changes in glomerular permeability in hypertension were determined using the osmotic pump method to measure the fractional clearance (Fc) of [3H]Ficolls of radii 36 Å, corresponding to the radii of albumin, transferrin, and immunoglobulin G, respectively. The Fc of [14C]transferrin and [14C]albumin was also determined as a marker of net transferrin and albumin clearance, respectively.

Methods

Experimental Animals

Male SHR (n=24) and WKY (n=24) weighing 200 to 250g were obtained from the Australian Resource Center (Perth, Australia). Rats were housed in rat boxes, under a 12-hour/12-hour day/night cycle with free access to standard rat chow and water for 8, 16, or 24 weeks. Systolic blood pressure was measured by tail-cuff plethysmography in conscious warmed rats.

Radiolabeling of Proteins

Rat serum albumin (RSA, Sigma Chemical Co) and rat transferrin (Sigma Chemical Co) were carbon-14 labeled with 125 μCi [14C]formaldehyde (NEN Life Science Products) using a modified reductive methylation technique. Polydisperse Ficoll-70 (Sigma Chemical Co) and dextran sulfate MW 48,000 (16% sulfur; degree of substitution, 1.7 sulfate groups per sugar residue; TdB Consultancy) were tritiated with sodium boro-[3H]hydride (NEN Chemical Co) using a modified reductive methylation technique.

Osmotic Pump Implantation for In Vivo Fc Studies of Albumin, Transferrin, and Ficoll

ALZET osmotic pumps Model 2001 (Alza PharmaceuticaI) were used to determine fractional clearances of [14C]albumin, [14C]transferrin, or [3H]Ficoll 70 as previously described. Rats were anesthetized by Forthane (Abbott Australasia Pty Ltd) inhalation. Blood and urine samples were analyzed for radioactivity as previously described. Glomerular filtration rate (GFR) was determined by creatinine assay.

Column Chromatography

[14C]Albumin in plasma and urine samples was analyzed using a Sephadex G-50 column (column dimensions, 18 cm x 1 cm; Pharmacia Fine Chemicals). [14C]Ficoll-70 in plasma and urine samples was fractionated using an S-300 column (column dimensions, 62 cm x 1.6 cm; Pharmacia Fine Chemicals). The run conditions have been described previously. The K_v of [3H]Ficoll corresponding to these radii was determined by calculating the elution of [3H]Ficoll (dpm/mL) at the corresponding K_v in urine and plasma samples.

Analysis of Dextran Sulfate Desulfation

Two milligrams [3H]dextran sulfate was injected intravenously into 24-week rats. Three-hour urine and corresponding plasma sample was taken and analyzed via ion exchange chromatography using a 19 cm x 1 cm² Q-Sepharose column (Pharmacia Fine Chemicals). The sample was applied in 6 mol/L urea, 0.05 mol/L Tris, 0.05% CHAPS, and 0.15 mol/L NaCl (pH 7.0) and eluted with a linear gradient of 1 mol/L to 2.5 mol/L NaCl in the same buffer. The percent intact dextran sulfate was determined using the area under the curve for intact dextran sulfate compared with total dextran sulfate elution.

Albumin Radioimmunoassay

[125I]-labeled RSA was prepared using the chloramine T method. The urinary albumin concentration was measured using a double antibody RIA. The interassay coefficient variation was 7% at a concentration of 180 μg/L. The detection limit of the assay was 31.2 μg/L.

RT-PCR

cDNA was synthesized with the Superscript First Strand synthesis system for RT-PCR ( GibCO BRL). Renal TGF-β1 and β-globin mRNA expression were analyzed by real-time quantitative RT-PCR (TaqMan system) as previously described. Primers and TaqMan probe for TGF-β1 and the endogenous reference 18S RNA were constructed by Primer Express (ABI Prism 7700, Perkin-Elmer Inc): TGF-β1 forward primer, 5' TTGCCCTCTACAACCAACAAACAA-3'; reverse primer, 5'-GGCTTGCGACCCACGTAGTA-3'. The TGF-β1-specific probe was FAM'-CCGGGTGCTTCCGATCATCACC-3'-TAMRA; FAM is 6-carboxyfluroscein; TAMRA (quencher) is 6-carboxy-tetramethylrhodamine. β-globin forward primer was 5'-TCCGCATTTGAAAACAGCTGCAT-3'; reverse primer, 5'-TCCACCTCGGTTTGATGCGAT-3'. β-globin-specific probe was FAM'-CCAGGGTTCCATACCGTTCCTTGTG-3'-TAMRA. Results expressed relative to control kidneys, which were arbitrarily assigned a value of 1.

Statistics

All data are expressed as mean±SD, where n is the number of determinations. The Student's t test was used to determine probability where P<0.05 was taken as statistically significant.

Equations

Fc was determined using the following equation: Fc=[(urinary albumin/plasma albumin) x (UFR/GFR)], where UFR is the urine flow rate (mL/min).

Total albumin (intact plus fragments) excretion was calculated using the following equation: [plasma albumin (in mg/mL)]/plasma albumin (in dpm/mL) x [urine albumin (intact plus fragments, in dpm/mL)] x [urine volume (in mL/24h)], where plasma albumin (mg/mL) is determined by RIA.

Percent intact albumin was determined using the following equation: RIA intact albumin (in mg/24h)/total albumin (in mg/24h) x 100/1.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.
Results

Physiological Parameters
The results in the Table demonstrate significant increases in systolic blood pressure in SHR at all time points in comparison to normotensive WKY. There was also no significant difference in the GFR between WKY and SHR at any time point.

Structural Integrity of Urinary \[^{14}C\]Albumin
A representative elution profile from Sephadex G-50 of \[^{14}C\]albumin in the urine and plasma of 24-week SHR and WKY is shown in Figure 1. The $K_v$ for albumin peptide elution is 0.89, and the $K_v$ for intact albumin elution is 0.13. Analysis of urine from WKY shows that most of the \[^{14}C\]albumin excreted is degraded to small fragments, with only 0.82%±0.63% (n=6) of the total albumin (intact plus fragments) being excreted intact (corresponding to an absolute excretion rate of 0.92±0.59 mg/24h of intact albumin, as determined by RIA). In comparison, the percentage of intact \[^{14}C\]albumin excreted by SHR increases to 6.82%±3.97% (10.05±4.2 mg/24h of intact albumin by RIA, n=6). Plasma \[^{14}C\]albumin from both SHR and WKY remained 100% intact with no fragments present. The increased excretion rate of intact albumin would not appear to be due to hyperfiltration, as there was no significant difference in the GFR between WKY and SHR studied at 24 weeks (Table).

Glomerular Permeability
Ficoll has a globular structure and is not reabsorbed by the tubules,26,27 so it represents a suitable model for the glomerular permeability of globular proteins. The results in Figure 2 demonstrate that there is no significant change in glomerular permeability to molecules of radii 36 Å, 48 Å, or 55 Å in 24-week WKY (closed bars) and SHR (open bars). n=6 for each data point.

### Physiological Parameters Associated with SHR and WKY

<table>
<thead>
<tr>
<th>Group</th>
<th>Systolic Blood Pressure, mm Hg</th>
<th>GFR, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR 8 week</td>
<td>218.33±18.62*</td>
<td>3.02±0.77</td>
</tr>
<tr>
<td>SHR 16 week</td>
<td>227.00±7.87*</td>
<td>2.50±0.23</td>
</tr>
<tr>
<td>SHR 24 week</td>
<td>224.67±6.06*</td>
<td>2.74±0.64</td>
</tr>
<tr>
<td>WKY 8 week</td>
<td>149.33±9.85</td>
<td>2.34±0.19</td>
</tr>
<tr>
<td>WKY 16 week</td>
<td>149.00±18.70</td>
<td>2.78±0.23</td>
</tr>
<tr>
<td>WKY 24 week</td>
<td>150.33±5.28</td>
<td>2.60±0.96</td>
</tr>
</tbody>
</table>

Values are mean±SD. n=6 for each time point.

* $P<0.001$ for comparison of age-matched WKY vs SHR.

![Figure 1](http://hyper.ahajournals.org/)

Representative profiles from Sephadex G-50 column showing the structural integrity of urinary \[^{14}C\]albumin (•) and plasma \[^{14}C\]albumin (○) for 24-week SHR and WKY.

![Figure 2](http://hyper.ahajournals.org/)

Fractional clearance of \[^{3}H\]Ficoll radius 36 Å, \[^{3}H\]Ficoll radius 48 Å, and \[^{3}H\]Ficoll radius 55 Å in 24-week WKY (closed bars) and SHR (open bars). n=6 for each data point.
The Fc of intact albumin by RIA in the SHR increased over the 8- to 24-week period, giving rise to the apparent albuminuria. This increase was not accompanied by any increase in the Fc of total albumin (intact plus fragments as determined by radioactivity) over the same time period (Figure 3). Normotensive WKY showed only a slight increase in clearance of intact albumin over the 8- to 24-week period, consistent with aging. Similar results were obtained when examined in terms of excretion rates (Figure 4). The Fc or excretion rate obtained from radioactivity is (1) considerably greater than that obtained by RIA because of the dominance of the fragment population in the radioactive excretion and (2) lower than the corresponding Fc of Ficoll with the same hydrodynamic size (36Å radius). Similar findings were obtained with [14C]transferrin, the Fc of which at 24 weeks did not alter significantly with hypertension (WKY, $5.19 \times 10^{-3} \pm 4.16 \times 10^{-4}$; SHR, $6.52 \times 10^{-3} \pm 1.58 \times 10^{-3}$; n=6) but is still considerably lower than the Fc of Ficoll of equivalent molecular radius of 48 Å. The lack of change in the Fc of the radiolabeled proteins demonstrates that the combination of glomerular permeability and tubular reabsorption of these proteins is not affected in SHR. Thus, what is observed in hypertension is an increase in the amount of intact albumin excreted, giving rise to significant intact albuminuria, without any change in the net amount of albumin excreted.

**Lyosomal Activity**

The desulfation of dextran sulfate is affected by lysosomal sulfatases and may be considered as a marker of in vivo renal lysosomal activity. In 24-week WKY, the amount of intact urinary dextran sulfate is $2.76\% \pm 1.08\%$ (n=4; compared with intact albumin of $0.82\% \pm 0.63\%$), whereas in 24-week SHR, the amount of intact dextran sulfate is $6.75\% \pm 1.54\%$ (n=6, P<0.005; with intact albumin at $6.82\% \pm 3.97\%$). The increase in the amount of intact dextran sulfate in the urine of SHR, in parallel with the increased excretion of intact albumin provides further evidence that lysosomal processing is directly associated with the increased levels of intact albumin in the urine of hypertensive rats.

**Renal TGF-β1 and βig-h3 mRNA Expression**

Figure 5a shows an increase in renal TGF-β1 mRNA in the SHR group at all time points. Increased translation of TGF-β1 was confirmed by increased renal βig-h3 mRNA (Figure 5b) (expression of which is induced by increased TGF-β production). Renal TGF-β1 levels increased in all groups in parallel with increased intact albumin excretion and decreased lysosomal activity.

**Discussion**

A widely recognized mechanism of albuminuria associated with hypertension is that caused by glomerular hyperfiltration, which may increase albumin filtration and which in turn may exert toxic effects on renal cells. Present studies have demonstrated that although there were significant increases in systemic blood pressure in SHR, there was no corresponding increase in GFR. This would be consistent with observations that both the excretion rate and Fc of intact albumin plus albumin-derived fragments, as determined by radioactivity, do not change over the 24 weeks studied. This means that some other factor must be responsible for the increased intact albuminuria in hypertension.

It is unlikely that alterations in the permeability of the glomerular capillary wall could account for the observed increase in intact albuminuria. We have demonstrated in this study that changes in the permeability of the glomerular capillary wall were not apparent when Ficoll Fc was measured. Further, there was no change in the excretion rate and
The combination of glomerular permeability and tubular uptake was also not altered. This would also eliminate the possibility of a saturation of the lysosomal degradation pathway, giving rise to an overflow of intact albumin. In addition, we have previously established that glomerular charge selectivity is not a factor, as studies have demonstrated this to be negligible.8,31,32 This can be rationalized with previous studies of charge selectivity, in which charged transport probes have been shown to be biochemically altered during filtration and renal passage, resulting in profound effects on clearance measurements. Before excretion, dextran sulfate is desulfated,31 and charged proteins are degraded to peptides.3 In the SHR, the increase in the excretion of intact albumin and dextran sulfate in streptozotocin-induced diabetic rats.11 This increase could be inhibited by ramipril and aminoguanidine.11 Thus, what is observed in hypertension is an increase in the amount of intact albumin excreted giving rise to significant intact albuminuria, without any change in the net amount of albumin excreted.

The new finding of similar changes in the renal processing of albumin and dextran sulfate suggests that the changes in lysosomal processing represent a specific metabolic event closely linked to a rise in blood pressure. A possible linkage factor is the role of TGF-β. This growth factor is known to decrease lysosomal enzyme activities,16 with increased production of TGF-β by mesangial cells when subjected to stretching forces brought about by high intraglomerular pressures.13–15 Our results suggest that TGF-β production in hypertension may be playing a role in albuminuria through this mechanism, with results showing decreased lysosomal activity with an upregulation of TGF-β, resulting in increased intact albuminuria irrespective of changes in glomerular permeability and net albumin excretion. TGF-β increases have commonly been associated with kidney hypertrophy and TGF-β fibrogenic potential.33 Here we describe a new link between TGF-β and the onset of kidney dysfunction in hypertension as determined by albuminuria. This supports approaches that recognize TGF-β as a therapeutic target34 in nephrotic states.

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References
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