URINARY EXCRETION OF ALBUMIN

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Renal albumin handling

Modified from Birn and Christensen
Kidney Int. 69, 440-449, 2006
The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells: retrieval is disrupted in nephrotic states

Russo LM, Sandoval RM, McKee M, Osicka TM, Collins AB, Brown D, Molitoris BA, Comper WD
Kidney Int. 2007; 71 (6): 504-13

Impaired tubular uptake explains albuminuria in early diabetic nephropathy

Russo LM, Sandoval RM, Campos SB, Molitoris BA, Comper WD, Brown D

Multiple factors influence glomerular albumin permeability in rats

Glomerular filtration barrier

Christensen et al. Seldin and Giebisch’s The Kidney. 2013
The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells. Russo et al. Kidney Int. 71, 504-513, 2007 GSC ~ 0.034

“Striations containing the green albumin are seen to extend from the basolateral to apical side.”

High capacity, low affinity uptake results in transcytosis.

Low capacity, high affinity (receptor mediated) uptake results in lysosomal degradation.
Measurement of GSC with Internal or External Detectors

Internal detectors (Zeiss and Olympus microscopes):
- Dextran 2,000,000 GSC = 0.000 ± 0.001, n = 8 glomeruli
- Dextran 2,000,000 GSC = 0.000 ± 0.001, n = 3 glomeruli
- Thyroglobulin GSC = 0.000, 0.001, n = 2 glomeruli

External detectors (Olympus microscope):
- Dextran 2,000,000 GSC = 0.005 ± 0.002, n = 5 glomeruli
- Thyroglobulin GSC = 0.023 ± 0.020, n = 5 glomeruli
- Rat serum albumin GSC = 0.013 ± 0.008, n = 10 glomeruli

Conclusion:
Use of external detectors leads to erroneously high glomerular sieving coefficients for large molecules.
### Table 1: Comparison of the glomerular albumin-sieving coefficient (SC) values.

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Method</th>
<th>SC</th>
<th>Species/animal model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tojo and Endou [12], 1992</td>
<td>Fractional micropuncture</td>
<td>0.00062</td>
<td>rat</td>
</tr>
<tr>
<td>Bertolatus and Hunsicker [62], 1985</td>
<td>131I-labeled BSA measuring urinary excretion and total kidney uptake minus interstitial nonfiltration uptake</td>
<td>0.0006</td>
<td>rat</td>
</tr>
<tr>
<td>Lund et al. [18], 2003</td>
<td>125I-native human serum albumin measuring both kidney uptake and urinary excretion</td>
<td>0.00066</td>
<td>rat</td>
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<tr>
<td>Norden et al. [13], 2001</td>
<td>urinary albumin excretion of congenital Fanconi syndrome patients</td>
<td>0.00008</td>
<td>human</td>
</tr>
<tr>
<td>Tencer et al. [63], 1998</td>
<td>Blockade of proximal tubular reabsorption by L-lysine</td>
<td>0.00033</td>
<td>rat</td>
</tr>
<tr>
<td>Christensen et al. [23], 2007</td>
<td>urinary albumin excretion of megalin-knockout mice</td>
<td>0.00016</td>
<td>megalin-knockout mice</td>
</tr>
<tr>
<td>Eppel et al. [14], 1999</td>
<td>tritium-labeled albumin</td>
<td>0.074</td>
<td>rat</td>
</tr>
<tr>
<td>Russo et al. [15], 2007</td>
<td>Alexa-labeled albumin, confocal microscopy</td>
<td>0.0341</td>
<td>rat</td>
</tr>
<tr>
<td>Tanner [16]</td>
<td>Alexa-labeled rat serum albumin, two-photon microscope with internal photodetectors</td>
<td>0.002</td>
<td>Munich-Wistar rat</td>
</tr>
</tbody>
</table>

GLOMERULAR FILTRATION AND URINARY EXCRETION OF ALBUMIN

Mouse, Rat

› Tojo, 1992, micropuncture in rat
› Comper, 2007, confocal microscopy in rat

Transformed to mice: Glomerular filtration 64 mg per 24 hours out of 35 mg circulating albumin

› Control mice: Urinary excretion 0.2 mg per 24 hours
› Meg KO mice: -II- 1.5 mg per 24 hours ~ GSC 1.6x10^{-4}
› Double (Meg/Cub) KO Mouse -II- 1.45 mg per 24 hours

Human

› Gekle: calculation from Compers figures in human kidney ~ 225 g filtered per 24 hours compared to a total of 125 g circulating albumin

› Lysine experiments in human kidney (Mogensen 1977) filtered and excreted ~ 400-500 mg per 24 hours ~ GSC ~ 6x10^{-5}
› Donnai-Barrow (megalin deficient) patients ~ 200 mg per 24 hours

› Normoalbuminuria < 30 mg per 24 hours
› Microalbuminuri 30-300 mg per 24 hours
› Nephrotic range albuminuria 3.5 g per 24 hours
Rat renal proximal tubule
Christensen et al.
Pflügers Arch. 2009
Albumin absorption and catabolism by isolated perfused proximal convoluted tubules of the rabbit

C. Hyung Park and Thomas Maack

J Clin Invest. 1984; 73: 767-77

Transcellular transport of intact $[^3H]C\text{Alb}$ was negligible

Absorbed Alb is hydrolyzed by tubular cells and catabolic products are readily released to the peritubular side
Basolateral secretion of reabsorbed nutrients and amino acids from the degradation of protein.
Renal handling of albumin in dogs with cubilin dysfunction


Urinary albumin excretion

Albumin/creatinine ratio (µg/mg)

- Normal dogs
- Affected dogs

RENAL ALBUMIN HANDLING IN MEGALIN KNOCK OUT MICE

Wt, Urinary excretion 0.2 mg/24 hours

Megalin KO, Urinary excretion 1.5 mg/24 hours

GSC in megalin KO mice: 0.00016
ENDOGENOUS ALBUMIN UPTAKE IN PROXIMAL TUBULE OF MOSAIC MEGALIN KNOCK OUT MICE
TRANSCYTOSIS OF ALBUMIN (RUSSO, MOLITORIS, COMPER ET AL. KIDNEY INT. 71, 504-13, 2007)

See commentary by M. Gekle
Kidney Int. 71, 479-81, 2007
General accepted GSC ~0.0005-0.0007
Mouse model of proximal tubule endocytic dysfunction

Kathrin Weyer, Tina Storm, Jingdong Shan, Seppo Vainio, Renata Kozyraki, Pierre J. Verroust, Erik I. Christensen, Rikke Nielsen

NDT, 26: 3446-51, 2011
Immunohistochemical labelling of the kidney cortex from **megalin-** and/or **cubilin-** deficient mice
INACTIVATION OF MEGALIN AND CUBILIN PROTEIN EXPRESSION USING A CRE TRANSGENE DRIVEN BY THE WNT4 PROMOTER
MEGALIN- AND CUBILIN-DEPENDENT UPTAKE OF ALBUMIN
URINARY EXCRETION OF ALBUMIN BY MEGALIN- AND/OR CUBILIN-DEFICIENT MICE

**A**

![Graph showing urinary albumin excretion](image1)

**B**

![Western blot analysis](image2)
Renal phenotypic investigations of megalin-deficient patients: novel insights into tubular proteinuria and albumin filtration

Tina Storm, Lisbeth Tranebjaerg, Carina Frykholm, Henrik Birn, Pierre J. Verroust, Tryggve Nevéus, Jens Michael Hertz, Erik I. Christensen, Rikke Nielsen.

NDT, 28: 585-591, 2013
Donnai-Barrow syndrome (DBS) is characterized by typical craniofacial features (ocular hypertelorism, enlarged fontanelle), ocular findings (high myopia, retinal detachment, progressive vision loss, and iris coloboma), sensorineural hearing loss, agenesis of the corpus callosum, intellectual disability, and congenital diaphragmatic hernia (CDH) and/or omphalocele. Low molecular weight proteinuria.
NOVEL SPLICE SITE MUTATION PRODUCES PREMATURE TRANSLATION STOP

A

B

control

[c.2639+1G>A]+[=]

non-affected mother

[c.2639+1G>A]+[=]

index patient

[c.2639+1G>A]+[c.2639+1G>A]
MEGALIN AND CUBILIN EXPRESSION IN CONTROL AND DB-PATIENT KIDNEY

Control  DB-patient

Megalin

Cubilin
URINARY PROTEIN EXCRETION IN THE PATIENTS
REDUCED TUBULAR REABSORPTION OF FILTERED VITAMIN D BINDING PROTEIN AND ALBUMIN IN MEGALIN DEFICIENT PROXIMAL TUBULES

Control  DB-patient

DBP

A

B

Albumin

C

D
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ALBUMIN ENDOCYTOSIS AND DEGRADATION IN VIVO 30 MIN AFTER I.V. DQ-ALBUMIN INJECTION

Albumin uptake in renal medulla of a megalin knockout mouse
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
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<tbody>
<tr>
<td>Pierre J. Verroust</td>
<td>INSERM UMR S968, Institut de la Vision, Paris, France</td>
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<td>Seppo Vainio</td>
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<td>Thomas Willnow</td>
<td>Max-Delbrück-Center for Molecular Medicine, Berlin</td>
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<td>Jens Michael Hertz</td>
<td>Department of Clinical Genetics, Odense University Hospital, Denmark</td>
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<tr>
<td>Rikke Nielsen</td>
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<td>Tina Storm</td>
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