OBJECTIVES

- Prevalence of obesity and obesity-associated Hypertension and CKD
- Effects of obesity on the kidney
- Overview of biomarkers to track kidney disease
- Focus on biomarker discovery for CKD
- Hurdles along the path and future directions
- Pilot study- MicroRNA in obesity and hypertension

THE OBESITY EPIDEMIC

Despite awareness campaigns and national resources being diverted to this problem, we have, at best, held steady.

- 31.8% of the sampled populations from NHANES study still remain overweight or obese.
- Boys and girls are almost equally prone to being obese.
- But does it matter how old they are?

THE OBESITY EPIDEMIC

We have made some progress in the toddler and young child demographic.

- Obesity rates of teenagers have risen over this period.
- Are all teenagers at the same risk?
**The Obesity Epidemic**


<table>
<thead>
<tr>
<th>Sex</th>
<th>Non-Hispanic</th>
<th>Hispanic</th>
<th>American</th>
<th>Non-Hispanic</th>
<th>Hispanic</th>
<th>American</th>
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</thead>
<tbody>
<tr>
<td>Race/ethnicity</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>White</td>
<td>148 (14)</td>
<td>148 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
</tr>
<tr>
<td>Black</td>
<td>232 (15)</td>
<td>232 (15)</td>
<td>193 (13)</td>
<td>193 (13)</td>
<td>193 (13)</td>
<td>193 (13)</td>
</tr>
<tr>
<td>Asian</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
<td>143 (14)</td>
</tr>
</tbody>
</table>

By Carol P. Fryer, M.P.H., Margaret S. Currie, M.P.H., and Cynthia C. Grimes, M.D., D.Sc.

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**Hypertension Prevalence**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Percent, No.</th>
<th>High BP</th>
<th>Moderate Risk BP</th>
<th>Either High or Moderate Risk BP</th>
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<tbody>
<tr>
<td>Male</td>
<td>415</td>
<td>13.0</td>
<td>8.6</td>
<td>21.6 (20.3)</td>
</tr>
<tr>
<td>Female</td>
<td>625</td>
<td>18.0</td>
<td>5.4</td>
<td>23.4 (22.6)</td>
</tr>
</tbody>
</table>


NAPRTCS Data

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**CKD Prevalence**

Epidemiology of chronic kidney disease in children


NAPRTCS Data
**CONCLUSIONS**

- CKD is fairly underreported in children.
- Consensus on definitions was lacking until recently, thus data cannot be compared.
- NAPRTCS data reported on causes with a large subcategory where no cause was immediately apparent/miscellaneous – 24% Could some of this be obesity associated CKD?
- There is a need to define the problem better on a large scale.

**OBESITY AND THE KIDNEY**

Mechanisms of Obesity-Associated Cardiovascular and Renal Disease

- Increased sodium reabsorption and hypertension mediated by sympathetic activity, RAS and intrarenal compression forces.
- Sympathetic activity mediated by genetic factors.
- Jackson heart study looking at relationship between leptin and blood pressure in AA.
- Early glomerular hyperfiltration and glomerulonegaly even without diabetes.
- FSGS.

**DEVELOPMENT OF MARKERS**

<table>
<thead>
<tr>
<th>Period</th>
<th>ACS</th>
<th>AKI</th>
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<tbody>
<tr>
<td>1960s</td>
<td>LDH</td>
<td>Serum creatinine</td>
</tr>
<tr>
<td>1970s</td>
<td>CK, myoglobin</td>
<td>Serum creatinine</td>
</tr>
<tr>
<td>1980s</td>
<td>CK-MB</td>
<td>Serum creatinine</td>
</tr>
<tr>
<td>1990s</td>
<td>Troponin T</td>
<td>Serum creatinine</td>
</tr>
<tr>
<td>2000s</td>
<td>Troponin I</td>
<td>Serum creatinine</td>
</tr>
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</table>

**Cystatin C**

- 13KDa protein Synthesized and released into plasma by all nucleated cells.
  - Still dependent on lean body mass.
- Serum cystatin C freely filtered (small and non-ionic).
  - More sensitive than Scr as marker of GFR.
- Cystatin C catabolized in PT.
  - Tubular damage ➔ appearance in urine.
BIOMARKER UTILITY
- Characteristic that can be objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic response to therapeutic interventions
- Could predict an outcome much earlier than traditional endpoints like survival

CURRENT BIOMARKERS OF GFR
Commonly used end points as measures of CKD progression
- Doubling of serum creatinine
- Decline in GFR by 50%
- Need for dialysis or kidney transplantation
- Yearly or monthly decline in GFR
- Worsening of proteinuria
- Graft loss

CKD BIOMARKERS
Characteristics of Promising New CKD Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sample</th>
<th>Origin</th>
<th>Biological function</th>
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</thead>
<tbody>
<tr>
<td>NGAL</td>
<td>urine</td>
<td>Direct detection Peptide with roles in survival and proliferation, binds metal ions</td>
<td></td>
</tr>
<tr>
<td>EN1</td>
<td>plasma</td>
<td>Personal proteome Peptide in urogenital system, regulates cell survival</td>
<td></td>
</tr>
<tr>
<td>L-FABP</td>
<td>urine</td>
<td>Personal proteome Peptide in urogenital system, regulates cell survival</td>
<td></td>
</tr>
<tr>
<td>NAG</td>
<td>urine</td>
<td>Glucose reabsorption in excretion of renal tubular cells</td>
<td></td>
</tr>
<tr>
<td>NGAL</td>
<td>blood</td>
<td>Liver, brain, pancreas, muscle, immune cells</td>
<td></td>
</tr>
<tr>
<td>L-FABP</td>
<td>blood</td>
<td>Personal proteome Peptide in urogenital system, regulates cell survival</td>
<td></td>
</tr>
<tr>
<td>ADMA</td>
<td>urine</td>
<td>Aldosterone, mineralocorticoid, anti-inflammatory, anti-angiogenic</td>
<td></td>
</tr>
<tr>
<td>APR-1</td>
<td>blood</td>
<td>Immune cells, regulators of angiogenesis, inhibits growth of TNF, VEGF</td>
<td></td>
</tr>
</tbody>
</table>

CATEGORIZATION
- Functional biomarkers- microalbumin, serum creatinine, serum Cystatin C
- Upregulated proteins- Neutrophil gelatinase-associated lipocalin (NGAL), Kidney injury molecule 1 (KIM-1), Liver-type fatty acid–binding protein (L-FABP), Interleukin 18 (IL-18), β-trace protein (BTP), Asymmetric dimethylarginine (ADMA)
- LMW proteins- Urine Cystatin
- Enzymes- N-acetyl-glucosaminidase (NAG), Glutathione-s-transferase (GST), gamma-glutamyl transpeptidase (GGT), Alanine aminopeptidase (AAP), Lactate dehydrogenase (LDH)
- Genetic markers- MicroRNA
MICROALBUMINURIA
- Early marker of CKD even in non diabetic patients
- Common in obese children and adolescents
- Measurement errors
  - orthostatic differences
  - validity of albumin/creatinine ratio
- Some studies show no difference between normal BMI and obese children- not universally ratified

URINARY NGAL
- Neutrophil gelatinase-associated lipocalin (NGAL), a 25-kDa protein, is known to be released from injured renal tubular cells in acute kidney injury (AKI) before a decrease in GFR can be detected
- In patients with moderate kidney disease, serum and urine NGAL concentrations have been identified to be independent predictors of CKD progression
- No difference noted in obese and normal BMI children

DIAGNOSTICS
The NGAL Test™ is available for most popular analyzers such as:
- Roche Cobas c501/c502 (Cobas 6000)
- Roche Hitachi Modular P
- Hitachi 917
- Beckman Coulter UniCel DxC
- Beckman Coulter (Olympus) AU640
- Abbott Architect
- Abbott Aeroset
- Siemens ADVIA 1800
- Siemens ADVIA 2400
- Siemens Dimension Vista
- Applications in development - Beckman Coulter AU5800

KIDNEY INJURY MOLECULE-1
- KIM-1 is a transmembrane glycoprotein, and its protein expression is not detectable in the normal kidney.
- The soluble form of KIM-1 can be easily detected in the urine of patients with AKI and can be used as an important biomarker for tubular injury
- Obese children had a higher urinary KIM-1 level compared with healthy controls
- KIM-1 may be a potential screening biomarker for the detection of early renal injury in obese children.

But....
- Patent pending as of January 2015 in the US.
- 19 countries already have it.

N-ACETYL-BETA-D-GLUCOSAMINIDASE
- NAG, a lysosomal enzyme found predominantly in proximal tubules, is normally excreted in low amounts as a consequence of the physiological exocytosis process.
- Early urinary marker for tubular cell malfunction
- Urinary NAG was significantly elevated in obese children compared with lean controls
- Also reproducible in mouse models with obesity
**Beta Trace Protein**
- BTP, also known as lipocalin prostaglandin D2 synthase, is a lipocalin glycoprotein.
- In a report from the mild to moderate kidney disease (MMKD) study group, BTP provided a reliable risk prediction for CKD progression.
- In a study of more than 800 African Americans with hypertensive CKD, higher BPT level was strongly associated with progression to end-stage renal disease (ESRD), compared with traditional markers of kidney function such as measured glomerular filtration rate (GFR).
- Although promising, BTP requires validation in large CKD populations.

**Future Possibilities**
- Ongoing discoveries in the field of proteomics, peptidomics, urinary transcriptomics and micro RNA analysis continue to reveal new candidates as biomarkers of CKD.
- Each candidate has to go through a long process of validation before FDA approval.
- CKD biomarker discovery being led by a consortium nationally.
- Obesity related renal injury has been recognized only recently and needs further population based demographical data as well as longitudinal studies of biomarkers.

**MicroRNAs in Obesity and Hypertension**
- Ongoing Pilot study in the Division of Nephrology.
- Regional consortium on biomarkers.
- National consortium on CKD biomarkers.

**Why is this Exciting News?**
- Urinary NAG and KIM-1 were not significantly different in obese children when co morbidities such as impaired glucose tolerance, insulin resistance, and hypertension were considered (Goknar, 2015 #6873).
- These two urinary markers could be specific for obesity associated renal injury.
- Longitudinal observational studies are needed to see if these tubular injury markers serve as biomarkers for obesity related renal injury.
MICRO-RNAs IN OBESITY AND HYPERTENSION

Serum RNA sequencing to develop a MicroRNA Biomarker for Obese Children with Hypertension

Scott Saint-Amour, MD
Fellow, Division of Pediatric Nephrology
Women and Children’s Hospital of Buffalo

MICRO-RNA

- MicroRNAs (miRNAs) are small (18-24 nucleotides) non-coding RNAs that regulate protein translation
  - Implicated in the pathways for:
    - Cellular repair and inflammation
    - Regulation of metabolic tissue development
    - Lipid Metabolism
    - Glucose homeostasis
    - Regulation of Renal Ion Transport
      - Preliminary studies show a variation in response to food intake

HYPOTHESIS

- Serum Micro-RNAs can serve as a biomarker for obesity associated vascular endothelial inflammation and hypertension.
  - In clinical practice up to 30% of obese children develop hypertension
  - Great need for an early biomarker that could indicate progression of disease toward hypertension in this population
    - Can lead to early intervention and reduction in associated morbidity
  - Further study into miRNA could reveal its use as a predictor, establishing risk of developing kidney disease
  - Understanding miRNA could also reveal new mechanistic insights into the complex regulatory networks underlying kidney disease, hypertension, and obesity.

STUDY DESIGN

- Blood samples from pediatric patients ages 3-18 have been collected and collated into 4 groups based on the presence/absence of both hypertension and obesity.
  - Control group negative for hypertension and negative for obesity
  - No subgroups for race/gender/ethnicity
- Exclusion Criteria
  - Pre-existing kidney disease or genetic disease
  - Diabetic nephropathy

miRNA ASSAY

- To be done in conjunction with the Thoracic Surgery Research Laboratory at Roswell Park
  - Sequencing library to be prepared with the TruSeq Small RNA kit from 1ug total RNA
  - Processed sequences are imported into CLC Genomics Workbench (An online comprehensive analysis package for analyzing, comparing, and visualizing sequencing data) and miRBase (a searchable database of published miRNA sequences and annotation)
    - http://www.mirbase.org/

ANALYSIS

- Currently, there are no known kidney-specific miRNAs that have been reported
  - We will use our preliminary data to establish the pattern of miRNA known to be involved in vascular injury and repair, hypertension, and cell inflammation, and document the differences between children with hypertension and without
  - We hope to establish baseline miRNA levels in each treatment group to examine if a statistically significant link between miRNA levels and hypertension and/or obesity exist
THE FUTURE

- This study aims to assess the possibility of establishing a laboratory evaluation that can be used to examine a child’s risk of developing complications due to hypertension and obesity, and their need for further, targeted, medical interventions.

REFERENCES