Renin

From a Laboratory Perspective

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Renin

- Protopolytic enzyme, 40kD MW
- Synthesized as prorenin
- Stored in granules as prorenin or renin
- Juxtaglomerular cells of the kidney
- Blood: prorenin to renin ratio: 10 to 1
- Prorenin is inactive
- Activation by enzymatic cleavage of prosegment
- Renin secretion tightly controlled

Use of renin and aldosterone measurements

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Expected findings</th>
<th>Renin</th>
<th>Aldosterone</th>
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<td>PA</td>
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<td>Renal artery stenosis</td>
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<td>Bartter’s syndrome</td>
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<tr>
<td>Secondary aldosteron deficiency</td>
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<td>Congenital adrenal hyperplasia</td>
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<td>Renin to monitor fludrocortisone treatment</td>
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</table>

Disorder

Expected findings

Guideline PA

- Patients with hypertension that are at increased risk for PA
- Use ARR to detect cases
- Conduct confirmatory testing
- If surgery desired
- If surgery not desired
- Subtype testing
- Treat with fludrocortisone
- Treat with laparoscopic adrenalectomy
Effect of medication on renin levels

**Table 6.** Factors that may affect the aldosterone-renin ratio and thus lead to false positive or false negative results.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Effect on aldosterone levels</th>
<th>Effect on renin levels</th>
<th>Effect on ARR</th>
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<tbody>
<tr>
<td>Betablockers</td>
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<td>t (FP)</td>
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<tr>
<td>Central alpha 2 agonists</td>
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<td>Angiotensin converting enzyme inhibitors</td>
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<td>NSAIDs</td>
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<td>Ke-urine diuretics</td>
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<tr>
<td>ARBs</td>
<td>d</td>
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<td>t (FP)</td>
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<td>CoQ10 (3HPh)</td>
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<td>t</td>
<td>t (FP)</td>
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<tr>
<td>Renin inhibitors</td>
<td>d</td>
<td>d</td>
<td>t (FP)</td>
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</tbody>
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Preanalytical conditions effecting renin levels

<table>
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<tr>
<th>Factor</th>
<th>Effect on renin level</th>
<th>Effect on ARR</th>
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</thead>
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<td>Posture</td>
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<td>Diet</td>
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<tr>
<td>Chronic kidney disease</td>
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<td>t (FP)</td>
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</table>
Plasma Renin Activity

**PRA assay**

- **ANGIOTENSINOGEN**
- **ANGIOTENSIN I**
- **ANGIOTENSIN II**
  - Enzyme inhibitor: PMSF
  - Correct for blank: 90 min 4 °C

**Endogeneous angiotensinogen as substrate**
**Low renin concentrations difficult?**
**Prolongation incubation time (37°C) may increase sensitivity**
**Lack of standardization**
  - Preanalytical conditions
  - Incubation time
  - pH
  - Differences in assay sensitivity
  - Precision/accuracy/reproducibility

**Renin mass assay**
**Direct Renin concentration (DRC)**

9/27 10/27

11/27 12/27
**Direct Renin assay**

- Independent of plasma angiotensinogen levels
- Sample processing at RT
- Assay can be automated
- Relatively large sample volume required
- Calibrated against WHO IR 68/356
- Interlaboratory CVs are lower than for PRA
- Analytical sensitivity?

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**EQAS (DRV) results**

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Preanalytical concerns II

- Proteolytic enzyme, 40kD MW
- Juxtaglomerular cells of the kidney
  - Synthesized as preprorenin
  - Stored in granules as prorenin or renin
  - Blood: prorenin to renin ratio: 10 to 1
  - Prorenin is inactive
  - Activation by enzymatic cleavage of prosegment
  - Renin secretion tightly controlled

 Activation of prorenin

- Proteolytic activation of prorenin
  - Irreversible
  - Proteolytic (serine proteases) removal of the prosegment
  - Secretory granules of the juxtaglomerular cells
  - Neutral or low pH (depending on proteases)
  - At low (4°C) temperature and neutral pH (cryoactivation)

- Nonproteolytic activation of prorenin
  - Reversible
  - Conformational changes (closed to open conformation)
  - At low (4°C) temperature (cryoactivation)

Effect of low temperature on PRA and DRC

Craven & Symonds, 1978

Schalkamp et al., 2008
Low renin - high prorenin!

- Blood: prorenin to renin: 10 to 1
- The lower the renin concentration the higher the proportion of plasma prorenin concentration
- Patients with low-renin hypertension or diabetic nephropathy have 100 fold more prorenin than renin levels

**Cryoadsorption**: falsely elevated renin values!
- 4 °C or lower, not at 37 °C, serum > EDTA plasma
- mainly due to conformational change of epitope
- Both PRA and DRC may be influenced

**Effect of Prorenin**

- [Graph showing effect of incubation temperature (22 °C, left and 37 °C, right) on assay (IRMA) performance]

**Activity or Mass?**

- Which assay is more elegant?
- Which assay is the fastest?
- Which assay is the most solid one?
- Which assay gives the best information?

**Reference Values for Aldosterone-Renin Ratios in Normotensive Individuals and Effect of Changes in Dietary Sodium Consumption**

Michiel N. Kerstens,1,* Anneke C. Muller Kobold,1 Marcel Volmer,2 Jan Koerts,2 Wim J. Sluiter,3 and Robin P.F. Dullaart1

- 100 normotensive individuals
- 50:50 m/f, age 20-70
- 10:10 per age decade
- PRA vs DRC (Cisbio)
- Aldosterone (Siemens RIA)
- Aldosterone-renin ratios
- Before & after 3 day oral salt loading test (additional 9 g NaCl/day)
**Reference Values for Aldosterone–Renin Ratios in Normotensive Individuals and Effect of Changes in Dietary Sodium Consumption**

Michael N. Kerstens,1 Anneke C. Müller Kobold,2 Marcel Walser,1 Jan Koerts,6 Wim J. Slater,1 and Robin P.F. Dullaart5

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**Precision profile, functional sensitivity and LOD**

**PRA vs DRC**

- **LOD = 0.45 nmol/L/h**
  - After SLT: 0.09-1.84 nmol/L/h

- **LOD = 0.7 ng/L**
  - FS = 1.0 ng/L
  - After SLT: 1.8-20.0 ng/L

**Passing Bablok:**
- **DRC ~ 10 x PRA**
- **LOD = 0.86 ng/L**
  - FS = 9.9 ng/L

**LOD = 0.7 ng/L**
- FS = 1.0 ng/L

**Comparison of reference values before and after sodium lowering therapy (SLT):**

<table>
<thead>
<tr>
<th>Aldosterone</th>
<th>Concentration (pmol/L)</th>
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<tr>
<td>Before SLT</td>
<td>After SLT</td>
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<tr>
<td>DRC</td>
<td>35 - 627</td>
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<td>PRA</td>
<td>0.1 - 2.35</td>
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<td>Aldosterone</td>
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<td>ARR</td>
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<td>8.7 - 54.3</td>
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<td>8.7 - 54.3</td>
<td>6.4 - 37.6</td>
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**Screening test**

**Confirmation test**

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Clinical Chemistry 52:11 (2011)
In conclusion

- Standardize preanalytical conditions
- Stop, if possible, antihypertensives
- Both PRA and DRC measure renin
- But PRA and DRC measure different things
- Prevent cryoactivation of prorenin
- PRA and DRC can both be influenced by cryoactivation
- Take caution when interpreting PRA or DRC results under antihypertensive medication (especially renin inhibitors)
- Reference values for DRC, Aldosterone, ARDrdc

Renin and units:

- pmol/L/min, ng/ml/h, mU/L, ng/L......

| Table 5: ARR cut-off values, depending on assay and based on whether PAC, PRA, and DRC are measured in conventional or SI units |
|---|---|---|---|
| PAC (μg/ml) | PRA (measured in pmol/L/min) | DRC² (measured in mU/L) | DRC* (measured in ng/L) |
| PAC (μg/ml) | 20 | 1 | 24 | 3.8 |
| | 40 | 3.1 | 3.7 | 3.7 |
| PRA (μmol/1) | 0.7 | 0.5 | 0.3 | 0.7 |
| | 0.5 | 0.3 | 0.5 | 0.7 |

Activity assays  Mass assays

Testosterone  renin  Ang I  ACE  Ang II  estrogen  AT1

Fig. 1. The figure displays the complex feedback loop of estrogen and testosterone interaction and the influence of estrogen and testosterone on various receptors.
Renin assay confusion…?

- **Enzyme kinetic (indirect) assay**
  - Plasma renin activity assay **PRA**
  - Endogenous substrate (angiotensinogen)
  - Plasma renin concentration assay **PRC**
  - Exogenous substrate (angiotensinogen)

- **Immunosorbent (direct assays)**
  - Immunoreactive renin
  - ELISA’s & IRMA’s

**Production Ang I**

**acTRC**

Total renin concentration (activity of prorenin & renin)

**irTRC**

Total renin concentration (concentration of prorenin & renin)


Renin assay confusion...?

- Enzyme kinetic (indirect) assay
  - Plasma renin activity assay \( \text{PRA} \)
  - Endogenous substrate (angiotensinogen)
  - Plasma renin concentration assay \( \text{PRC} \)
  - Exogenous substrate (angiotensinogen)
  - Total renin concentration (activity of prorenin & renin) \( \text{acTRC} \)

- Immunosorbent (direct assays)
  - Immunoreactive renin assay
  - Total renin concentration (concentration of prorenin & renin) \( \text{irTRC} \)

Schalekamp et al.

Campbell et al.
Clinical Chemistry 55:5
863–877 (2009)