1. The following is the abstract of a recent paper.

**Purpose:** Hyperoxaluria is a prominent risk factor for calcium oxalate urinary stones. Oxalate in urine is synthesized in the body or absorbed from food in the gastrointestinal tract. The amount of oxalate absorbed by patients with calcium oxalate stones may vary from a few percent to 50% of the dietary intake. Reference values for oxalate absorption measured under a standardized diet have never been attained in sufficient numbers from healthy individuals. Therefore, to our knowledge we collected for the first time the values required to interpret test results in patients with recurrent urinary stones.

**Materials and Methods:** A total of 120 healthy volunteers, including 60 females and 60 males, received an identical standard diet on 2 consecutive days. On the morning of day 2 a capsule containing 0.37 mmol sodium [C-13(2)]oxalate (not radioactive) was ingested with water. Urinary oxalate was measured by gas chromatography-mass spectrometry. Absorption at a fixed 800 mg. daily Ca input is expressed as a percent of the labelled oxalate dose.

**Results:** For the standardized [C-13(2)]oxalate absorption test the reference range in 95% of the 120 volunteers was 2.2% to 18.5% (mean ± SD 7.9% ± 4.0%). The repeatability of the standardized test was determined in 26 of the 120 volunteers by repeating the test twice. The mean intra-individual SD was 3.39% ± 1.68%.

**Conclusions:** We assessed reference values of intestinal oxalate absorption using a standardized diet. Inter-individual and intra-individual variance was high.


**Questions about this report**

a) What do they mean by ‘reference range’?

b) What method do you think they have used to calculate the reference range?

**N.B.** I have no idea what they mean by ‘The mean intra-individual SD was 3.39% ± 1.68%.’

2. The following is the abstract of a recent paper.

**Background:** The measurement of urinary free cortisol (UFC) is commonly used in the investigation of possible Cushing’s syndrome. With the recent availability of liquid chromatography-tandem mass spectrometry (LC-MS/MS) in hospital laboratories, we wanted to develop a specific UFC LC-MS/MS method and compare it with our current immunoassay method and develop a new LC-MS/MS reference range if required.

**Methods:** A UFC LC-MS/MS method using deuterated cortisol as an internal standard was optimized using solid-phase extraction as a clean-up procedure. The
multiple reaction-monitoring transitions used for the detection of cortisol and deuterated cortisol were 363.1 > 121 and 365.1 > 121.8, respectively. The method was investigated regarding precision, linearity, sensitivity, recovery and interference. UFC was measured by the in-house urine adapted ACS:180 serum cortisol immunoassay and the developed LC-MS/MS method in 110 urine samples from patients being investigated for possible Cushing’s syndrome.

Results: The within-batch precisions (n = 25) of the LC-MS/MS method were 7.6%, 4.5% and 3.3% at 25.0 nmol/L, 49.6 nmol/L and 344.6 nmol/L, respectively; the between-batch precisions (n = 10) were 9.4%, 9.4% and 8.4%, respectively, at these concentrations. The method is sensitive down to 5 nmol/L and linear up to at least 1000 nmol/L. The method showed adequate cortisol recovery and no interference from the numerous drugs and steroids tested. The total run time for 20 samples, including sample preparation, was 120 min. A scatter plot of paired UFC measurements on the LC-MS/MS and the ACS:180 gave the equation: LC-MS/MS = 0.408 (ACS: 180) + 2.65, \( r^2 = 0.6664 \). The 24-h measured UFC results on 110 samples (25 men and 85 women) were positively skewed. After log transformation the data were less skewed, and following back transformation of the lower 97.5\(^{th}\) centile, the upper limit of normal was 165 nmol/24 h. The 95th centile of the untransformed data was 146 nmol/24 h (n = 110, 25 men and 85 women). Separated by sex, the 95th centile was 152 nmol/24 h for men (n = 25) and 141 nmol/24 h for women (n = 85).

Conclusions: We have developed a UFC LC-MS/MS method with a solid-phase extraction clean-up step. The method shows adequate performance and is suitable for routine laboratory use. The mixed sex (n = 110, men = 25, women = 85) reference range was up to 165 nmol/24 h or 146 nmol/24 h, depending on how the data are manipulated.


Questions about this report

a) The authors found a regression equation with the LC-MS/MS method of measuring UFC as the \( y \), dependent, or outcome variable and the ACS:180 method as the \( x \), independent, or predictor variable to be

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\text{LC-MS/MS} = 0.408 (\text{ACS: 180}) + 2.65
\]

What is the slope and intercept for this line? What do they tell us about the agreement between these two methods of measurement?

b) The correlation coefficient between the LC-MS/MS method and the ACS:180 method is given as the square, \( r^2 = 0.6664 \). This gives us \( r = 0.82 \). What does the correlation coefficient tell us about the agreement between these two methods of measurement?

c) Why were the data log transformed?

d) What method do you think they used to estimate the centiles?

e) What possible reason could they have for estimating the 97.5\(^{th}\) centile using the log transformed data and the 95th centile using the untransformed data?