Deacon's Challenge No. 85 Answer

A 35-year old woman was found to have a fasting plasma glucose concentration of 6.2 mmol/L. One week later, the baseline sample of an Oral Glucose Tolerance Test gave a glucose of 5.5 mmol/L. The Hoorn study in a largely elderly Caucasian population has shown that the biological coefficient of variation (CV) for fasting plasma glucose is 6.3% and your assay runs a standard deviation (SD) of 0.04 mmol/L, on its low control at 2.4 mmol/L. Is it likely that the patient was not fully fasting for the first sample? Justify your answer and state any assumptions you make.

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The difference between the two "fasting" plasma glucose concentrations is 6.2 – 5.5 = 0.7 mmol/L. This *difference* will hopefully be normally distributed and, if there is no real difference between the means of the two hypothetical sets of data to which they belong, the mean difference will be zero. It can be shown that if two individual results are not statistically significantly different (i.e. their *difference* falls within 95% confidence limits of a distribution for *differences* with mean zero) then their *difference* must be less than 2.8 *standard deviations*.

A problem which always arises in a question of this type is that the standard deviation (*SD*) and hence 2.8 *SD*, may not be the same at both plasma glucose concentrations which can sometimes lead to a different interpretation depending upon which value is used. The easiest way to get around this difficulty is to calculate the *SD* at the mean of both values [i.e. (6.2 + 5.5)/2 = 5.85 mmol/L].

Our task is therefore to determine a value for the standard deviation to be used. This should be the *total* SD calculated from the component *intra-individual* biological variation and *analytical* variation. The biological coefficient of variation (*CV*) quoted from the Hoorn study must reflect either *total* biological variation or more likely *inter*-individual biological variation whereas for a single patient it is *intra*-individual variation which is relevant. This difference will have to be ignored. The next step is to convert this biological *CV* to the *SD* at the mean plasma glucose of 5.85 mmol/L:

$$\text{Biological } SD \text{ at } 5.85 \text{ mmol/L} = \frac{(\text{Biological } CV \text{ at } 5.85 \text{ mmol/L}) \times 5.85}{100}$$

$$= \frac{6.3 \times 5.85}{100} = 0.37 \text{ mmol/L (2 sig figs)}$$

Another problem which occurs is that the analytical *SD* for the laboratory's glucose method is given at a concentration of 2.4 mmol/L and this may or may not apply at the observed plasma levels. This analytical *SD* can be corrected by assuming that the *SD* is proportional to concentration (i.e. that *CV* is constant) so that at the mean plasma glucose of 5.85 mmol/L the analytical *SD* is 0.04 x 5.85/2.4 = 0.10 mmol/L (2 sig figs).

The total *SD* can then be calculated as follows

$$SD_{Total}^2 = SD_{Biological}^2 + SD_{Analytical}^2$$

$$SD_{Total}^2 = 0.37^2 + 0.10^2$$

$$= 0.137 + 0.01 = 0.147$$

$$SD_{Total} = \sqrt{0.147} = 0.383 \text{ mmol/L}$$

$$2.8\ SD_{Total} = 2.8 \times 0.383 = 1.1 \text{ mmol/L (2 sig figs)}$$

Since the difference in the two plasma glucose concentrations (0.7 mmol/L) is much less than 2.8 *SD* there is no statistically significant difference (i.e. $P>0.05$) between them and it is quite likely that the patient fasted fully on both occasions.

N.B. even assuming that the analytical *SD* remains at 0.04 mmol/L, 2.8 *SD* becomes 1.0 mmol/L which makes no difference to the overall conclusion. If the *intra*-individual biological variation had been available then 2.8 *SD* would be even lower but it is impossible to predict what effect this would have).

Question 86

Lactate can be measured enzymatically by oxidation to pyruvate by lactate dehydrogenase (LDH) in the presence of NAD^+:

$$\text{L-lactate} + NAD^+ \xrightarrow{LDH} \text{Pyruvate} + NADH + H^+$$

NADH has a molar absorption coefficient of 6.22×10^3 $L.mol^{-1}.cm^{-1}$

Method

To 2.0 mL buffer, add 0.1 mL sample, 0.2 mL NAD^+ (27 mmol/L) and 30 µL LDH solution.

Result

The absorbance change (relative to a reagent blank, using a standard 1 cm cuvette) in the assay was 0.82.

Calculate the lactate concentration in this sample.

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