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31. Generally the bioavailability of dietary CoQ10 is low, and the formulations of CoQ10 supplements affect bioavailability (8, 9). There is also a significant difference between individuals in absorption of CoQ10 from supplements (9). Knowledge of the biological variation of CoQ10 in healthy individuals enables interpretation of whether a significant change has occurred in response to a disease state or supplementation.

32. Smith MW, Dean M, Carrington M, Winkler C, Huttley GA, Lomb DA, et al. CoQ10, 10 healthy adult male volunteers were enrolled. Exclusion criteria included taking CoQ10 or other vitamin supplements, smoking, and use of any medications within 4 weeks before initiation of the study. All participants were self-reportedly healthy and disease free throughout the study. The median age of the participants was 23.5 (range, 21–28) years, the median weight was 69 (60 –100) kg, and the median body mass index (BMI) was 21.4 (18.5–28.6) kg/m2. Blood samples were taken in the morning after a 10-h fast. Samples were taken at least 1 week apart, over a period of 2 months, with seven samples being taken in total. Blood samples were collected into evacuated glass tubes containing lithium heparin. Blood was immediately placed on ice and centrifuged within 1 h of collection; the resulting plasma was stored at −80 °C until analysis.


36. Ghiardi G, Blondi ML, Caputo M, Levi S, DeMonti M, Guagnellini E, et al. Coenzyme Q10 (CoQ10) is an essential cofactor in the mitochondrial electron transport chain and is found in all cell membranes. It is present in the body in both the reduced and oxidized forms, with the reduced form (CoQ10H2) having antioxidant properties. CoQ10 has been implicated in disease processes, including Parkinson disease (1), diabetes (2), and Alzheimer disease (3), as well as in aging (4), oxidative stress (4, 5), and hydroxymethylglutarate-CoA reductase inhibitor (statin) therapy (6). In particular, changes in CoQ10 may be relevant to statin-induced myalgia (7).

Biological Variation of Coenzyme Q10 Sarah L. Molyneux,2* Christopher M. Florkowski,1 Michael Lever,1,2 and Peter M. George1 (1) Canterbury Health Laboratories, Christchurch, New Zealand; (2) Department of Chemistry, University of Canterbury, Christchurch, New Zealand; * address correspondence to this author at: PO Box 151, Christchurch, 8000 New Zealand; fax 64-3-3640889, e-mail sarah.molyneux@cdhb.govt.nz)
For the reference interval study, 205 participants (90 males and 115 females) who were self-reportedly healthy and disease free were enrolled from the electoral role or from responses to advertisements. A questionnaire was used for screening into the study. The reference group comprised 90% New Zealand Europeans, 5% New Zealand European/Maori, and 5% other ethnicities. Eight percent of the group smoked one or more cigarettes per week, and 35% were taking vitamin supplements; however, none was taking any form of CoQ10 supplement. Median age was 44 (range, 18–83) years, median BMI was 25.6 (17.1–46.0) kg/m2, median LDL-C was 3.01 (1.37–6.55) mmol/L, and median TC was 5.54 (3.20–9.70) mmol/L. Blood samples were taken between 0750 and 1105 in the morning, with 115 participants having fasted overnight. Samples were collected into evacuated glass tubes containing lithium heparin. The blood was centrifuged within 1 h of collection, and the resulting plasma was stored at −30°C until analysis. These studies were approved by the Canterbury Ethics Committee, Christchurch, New Zealand. Written informed consent was obtained from all participants.

Total CoQ10 was measured by HPLC with electrochemical detection, using a method similar to that described by Tang et al. (10). The within- and between-run CVs for the total CoQ10 assay (determined by analyzing two samples in quadruplicate in six different runs) were −3.3%. Direct LDL-C was measured with the Roche Diagnostics reagent, with a CV of 1.2%. TC, triglycerides, and HDL-cholesterol (HDL-C) were measured by an enzymatic colorimetric method (Aerosef analyzer Model LN; Abbott Laboratories). The CVs for the TC, triglycerides, and HDL-C assays were 1.6%, 1.1%, and 5.3%, respectively.

Statistical analyses were conducted with SigmaStat and SPSS software (SPSS, Inc.). Variance estimates for the inter- and intraindividual and analytical variation were determined with a REML variance decomposition procedure and are expressed as CVs. Correlation analysis was performed with the Pearson correlation coefficient. Outliers were included in determination of the reference interval because the nonparametric analysis allows for these. Comparisons were performed with the Mann-Whitney rank-sum test. Statistical significance was inferred when \( P < 0.05 \).

For the 10 participants studied to investigate the biological variation of CoQ10, the median values (interquartile ranges) were 0.85 (0.66–0.99) µmol/L for total CoQ10; 2.75 (2.24–3.28) µmol/L for LDL-C; 169 (147–198) µmol/mol for the ratio of total CoQ10 to LDL-C; 4.75 (4.10–5.70) µmol/L for TC; 289 (252–348) µmol/mol for the ratio of total CoQ10 to TC; 1.20 (1.00–1.50) µmol/L for triglycerides; and 1.12 (0.99–1.31) µmol/L for LDL-C. The interindividual variations in total CoQ10, the ratio of total CoQ10 to LDL-C, and the ratio of total CoQ10 to TC were 29%, 26%, and 18%, respectively. The intraindividual variations for these variables were 12%, 15%, and 14%, respectively.

In the reference interval cohort, there were no significant differences in total CoQ10, LDL-C, ratio of CoQ10 to LDL-C, TC, or ratio of CoQ10 to TC for the fasting and nonfasting groups; data were therefore pooled. The distribution for total CoQ10 for the complete cohort was not gaussian, being skewed toward higher concentrations.

The 95% interfractile intervals for total CoQ10 and other ratios [stratified where appropriate (11, 12)] are shown in Table 1. There was a significant difference in total CoQ10 and the ratio of CoQ10 to TC between males and females (\( P = 0.008 \) and \( P < 0.001 \), respectively), which has been reported previously (13, 14). The 95% interfractile intervals for total CoQ10 for males and females (Table 1) are similar to those reported by Miles et al. (13).

On the basis of the criteria recommended by Harris and Boyd (11) and the NCCLS (12), the reference interval need not be stratified for total CoQ10 according to gender. There was a trend (\( P = 0.075 \)) for a difference in the ratio of CoQ10 to LDL-C between males and females, but it was not statistically significant.

We found a significant positive trend for total CoQ10, LDL-C, and TC to increase with increasing age (\( P < 0.001; r = 0.277, 0.385, \) and 0.439 respectively). This trend supports the finding of Kaikkonen et al. (14). In the present study, the correlation of CoQ10 and age disappeared when cholesterol was included in a multivariate analysis. Application of the criteria recommended by Harris and Boyd (11) and the NCCLS (12) indicated that separate reference intervals for total CoQ10 according to age are justified (Table 1).

There was a significant trend (\( P < 0.008 \)) for CoQ10, LDL-C, and TC to increase with increasing BMI (\( r = 0.246, 0.249, \) and 0.188, respectively). However, there is no case for stratifying the reference interval according to BMI (11, 12).

With knowledge of biological variation and analytical imprecision for the assay, it is possible to calculate a reference change value (RCV), or “critical difference”, for serial results to be significantly different (Eq. 1) (15).

\[
\text{RCV} = \sqrt{2} \times Z \times \sqrt{\frac{C_{V_a}^2}{2} + C_{V_i}^2}
\]

Where \( C_{V_a} \) is analytical imprecision and \( C_{V_i} \) is intraindividual variation. For change, it is customary to use bidirectional Z scores, with 95% probability regarded as significant and 99% as highly significant. The corresponding Z scores are 1.96 and 2.58, respectively. Thus, for total CoQ10, with a \( C_{V_i} \) of 12% and \( C_{V_a} \) of 3.3%, a 95% significant change is 35.0%, and a 99% significant change is 46.1%.

It is clear that CoQ10 results from different individuals are tightly distributed around a homeostatic setpoint and that significant changes in CoQ10 can occur within the reference interval. For example, with a starting CoQ10 concentration of 1.00 µmol/L, the concentration can decrease to 0.65 µmol/L (a 95% significant change) or 0.54 µmol/L (a 99% significant decrease) and still be within the reference interval.

The usefulness of reference intervals has been further addressed by the concept of biological individuality (16). This is expressed as the index of individuality described as the ratio of \( C_{V_i}/C_{V_a} \), which is the ratio of intraindivi-
Table 1. The 95% interfractile reference intervals for all analytes, ratios, and subgroups meriting stratification.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>95% interfractile reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CoQ10</td>
<td>205</td>
<td>0.46–1.78 μmol/L</td>
</tr>
<tr>
<td>Males*</td>
<td>90</td>
<td>0.45–2.05 μmol/L</td>
</tr>
<tr>
<td>Females*</td>
<td>115</td>
<td>0.46–1.71 μmol/L</td>
</tr>
<tr>
<td>Age 18–44 years</td>
<td>105</td>
<td>0.43–1.61 μmol/L</td>
</tr>
<tr>
<td>Age 45–83 years</td>
<td>100</td>
<td>0.57–1.95 μmol/L</td>
</tr>
<tr>
<td>LDL-C</td>
<td>205</td>
<td>1.50–4.98 mmol/L</td>
</tr>
<tr>
<td>Ratio of total CoQ10 to LDL-C</td>
<td>205</td>
<td>158–522 μmol/mol</td>
</tr>
<tr>
<td>TC</td>
<td>205</td>
<td>3.57–8.40 mmol/L</td>
</tr>
<tr>
<td>Ratio of total CoQ10 to TC</td>
<td>205</td>
<td>101–265 μmol/mol</td>
</tr>
<tr>
<td>Males</td>
<td>90</td>
<td>121–284 μmol/mol</td>
</tr>
<tr>
<td>Females</td>
<td>115</td>
<td>88–244 μmol/mol</td>
</tr>
</tbody>
</table>

* These subgroups are not statistically required to be stratified based on Harris and Boyd criteria (11), but they may be of interest.

vidual to interindividual variation (16). When the index is low, particularly when it is <0.6, the dispersion of values for any individual will span only a small part of the reference interval. Reference values will thus be of little use, in particular for deciding whether a significant change has occurred. Conversely, when the index is high, particularly when it is >1.4, values from a single individual will cover much of the entire distribution of the reference interval. In this context, reference values will be of significant value for clinical interpretation. For total CoQ10, the index of individuality is 12.2/29.0 = 0.42; thus, it is low.

In conclusion, we have derived data for the biological variation of CoQ10 together with reference values for the New Zealand population. The low index of individuality for CoQ10 indicates that individual CoQ10 concentrations are tightly distributed around a homeostatic setpoint and that significant changes can occur within the reference interval. From a statistical perspective, seven samples should be evaluated to ensure that the estimate of the homeostatic setpoint is within 10% of the true value, with 95% probability (15). From a clinical perspective, serial changes in CoQ10 should be evaluated against the RCV to allow for both biological variation and analytical imprecision.

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References

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Application of Commercial Calibrators for the Analysis of Immunosuppressant Drugs in Whole Blood, Thomas M. Annesley (University of Michigan Health Systems, 1500 East Medical Center Dr., Ann Arbor, MI 48109-0054; e-mail annesley@umich.edu)

Multiple immunosuppressant drugs are now available to prevent organ rejection. Because of their different modes of action, these drugs are often prescribed together as part of a multidrug protocol. This has created a challenge for clinical laboratories to analyze multiple immunosuppressant drugs in the same blood specimen. HPLC–mass spectrometry (HPLC-MS) or -tandem mass spectrometry (HPLC-MS/MS) (1–6) assays can measure all of the common drugs.

A practical issue with the use of HPLC-MS/MS is the necessity of preparing whole-blood calibrators containing cyclosporine, tacrolimus, and sirolimus. With the introduction of everolimus, yet another set of calibrators will be needed. As with any assay for which in-house calibrators are prepared, this added variable will contribute to the interlaboratory differences observed in proficiency testing programs. The availability of either standard reference materials (SRMs) or uniform calibrators for use