COENZYME Q10 IN HEALTH AND DISEASE

Canterbury Health Laboratories

PM George, RJ Mackay, Molyneux SL, Young JM, Lever M and Florkowski CM

Canterbury Health Laboratories

Canterbury District Health Board
Te Poari Hauora ō Waitaha

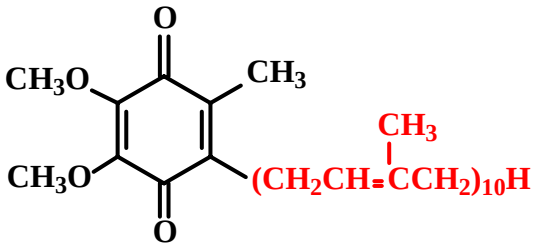
Presentation Outline

Canterbury Health Laboratories

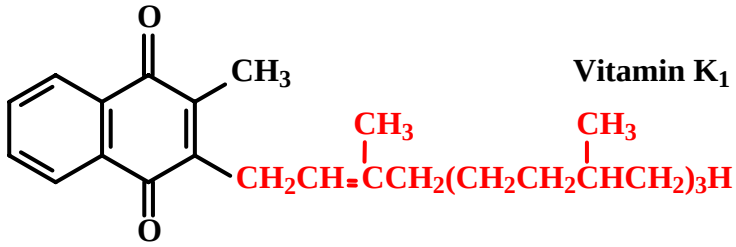
- Background on CoQ
- Measuring CoQ
- Normal values and variation
- CoQ in disease states
  - Statin therapy
  - Statin induced myalgia
  - Heart Failure
  - Endothelial function
  - Diabetes
  - Hypertension

CoQ10 and vitamin K

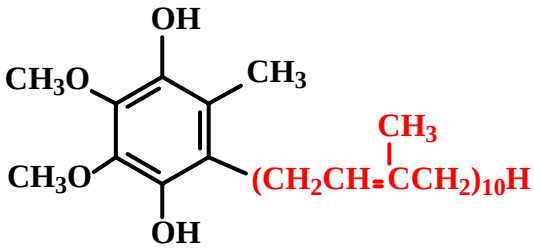
Canterbury Health Laboratories



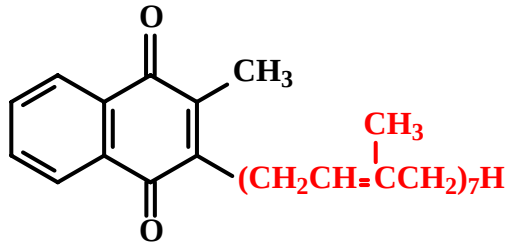
Ubiquinone
Coenzyme Q_{10}
CoQ_{10}



Vitamin K_1



Ubiquinol
Reduced Coenzyme Q_{10}
$CoQ_{10}H_2$



Vitamin K_2

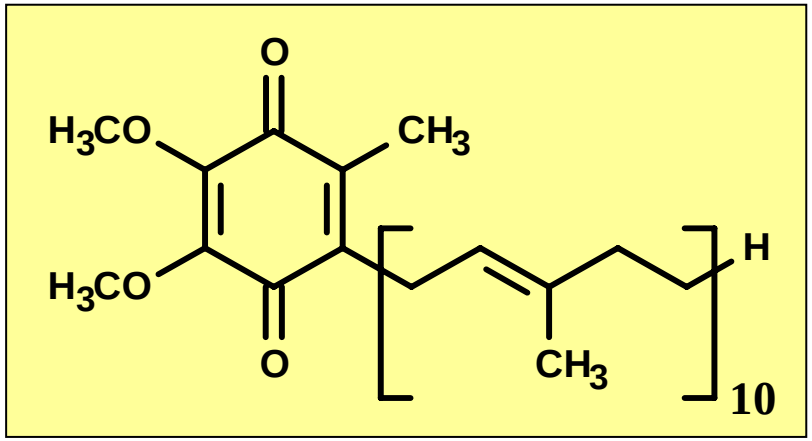
Coenzyme Q is a hydrophobic quinone (ubiquinone) involved in electron transport. In the body it occurs mainly as the hydroquinone (ubiquinol).

It is chemically related to the K vitamins, and similar analytical principles can be applied to both groups of compounds.

CoQ

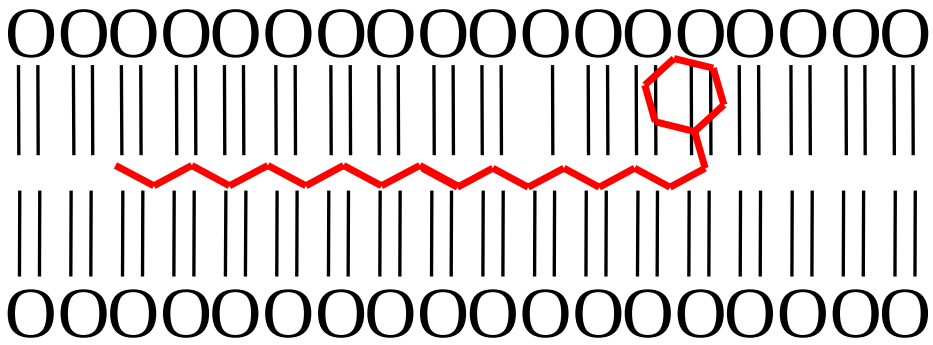
Canterbury Health Laboratories

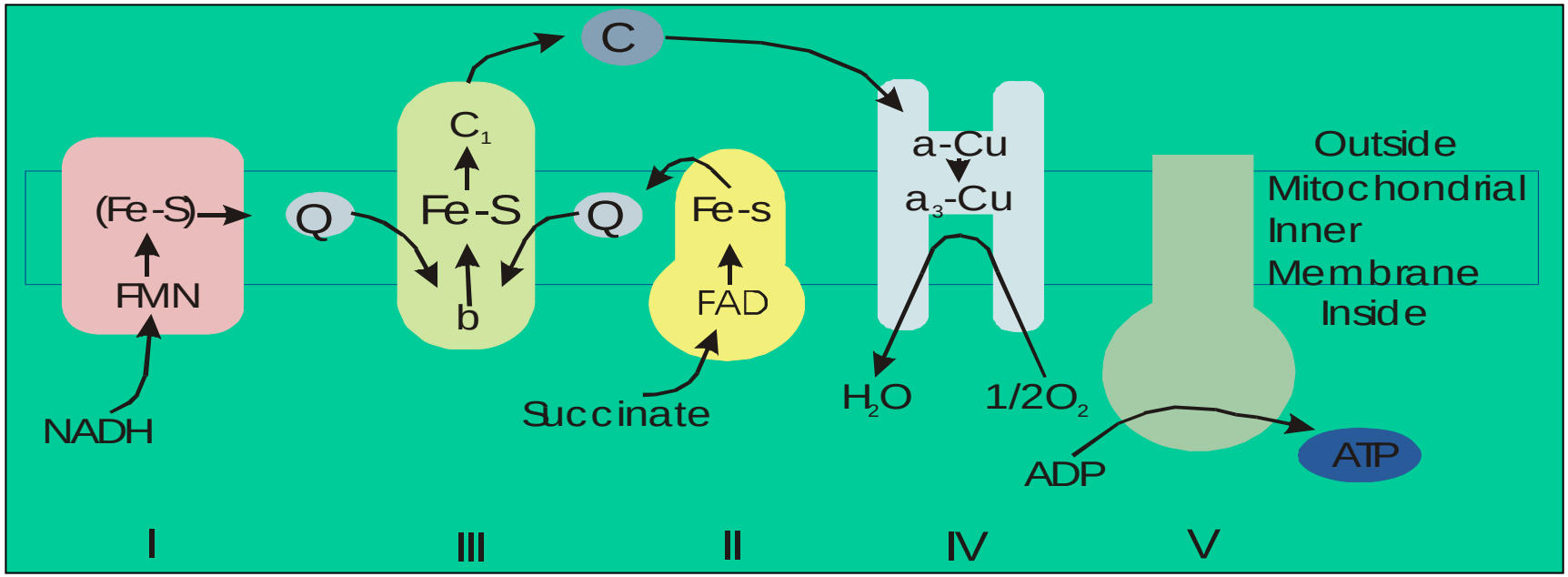
- Synthesised endogenously
- Present in the body in reduced and oxidised form , 96% is in reduced form in healthy subjects
- Reduced to oxidised ratio may indicate oxidative stress
- Reduced form can act as an antioxidant
- Circulates in blood bound to lipoproteins



CoQ - role in health

Canterbury Health Laboratories

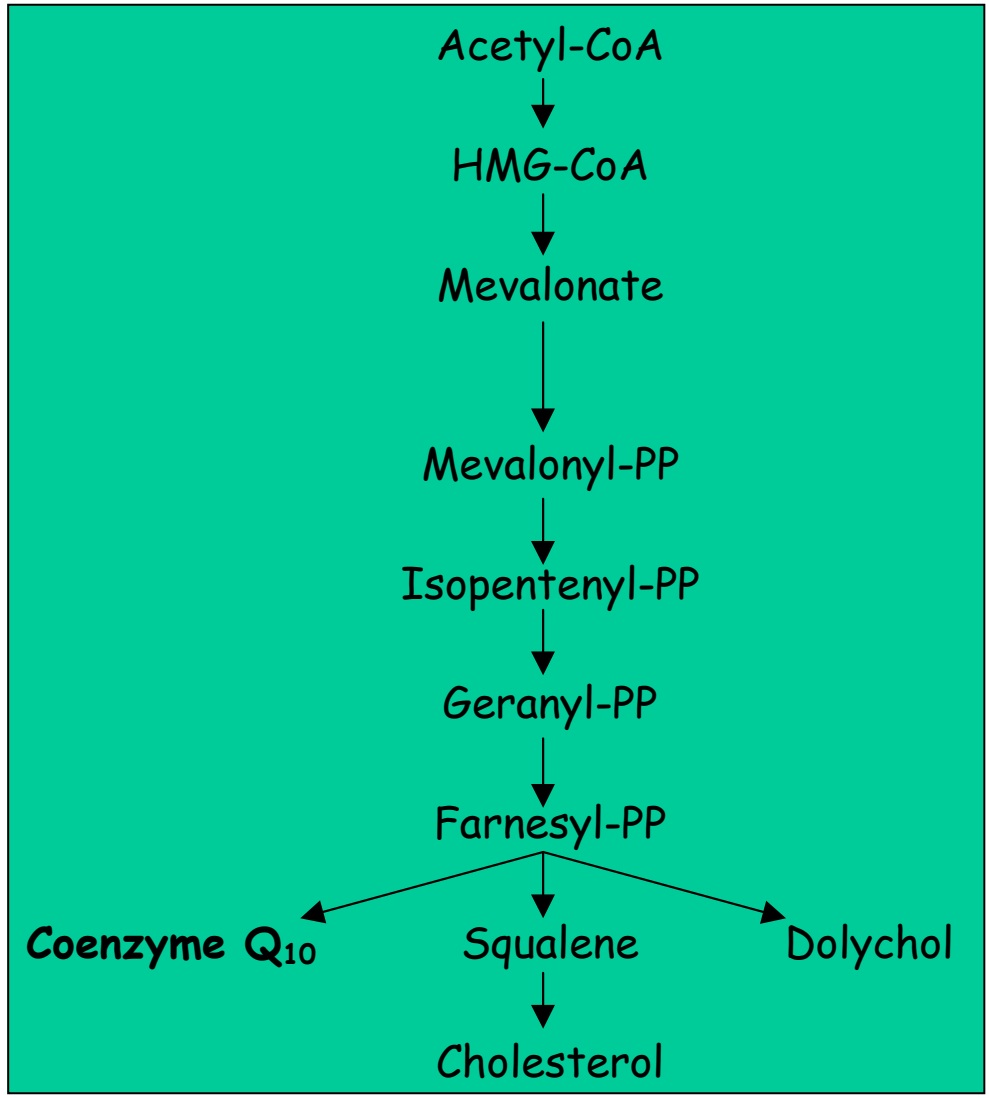




CoQ - role in health

Canterbury Health Laboratories

- Antioxidant
  - reduced form only
  - regenerates α-tocopherol
- Mitochondrial function
  - generation of ATP
- Synthesis by the mevalonate pathway
- Also obtained from the diet - especially meats



Why measure Coenzyme Q?

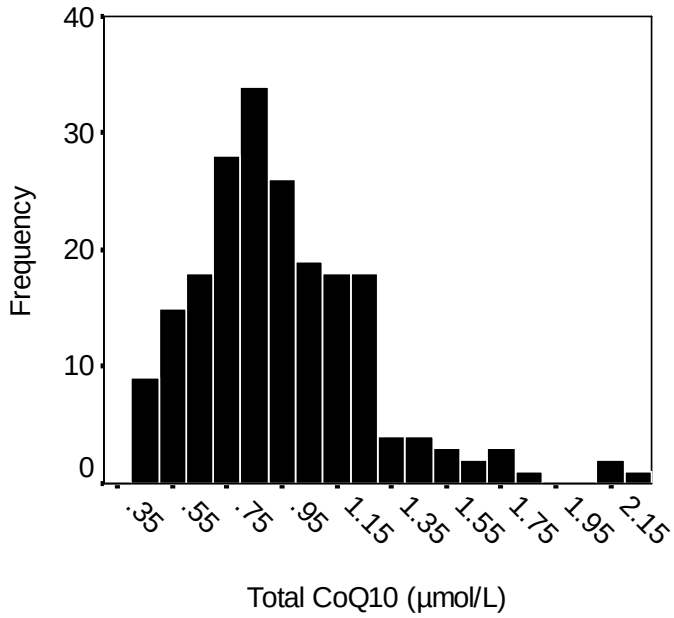
Canterbury Health Laboratories

- Coenzyme Q (CoQ) biosynthesis is inhibited by statin drugs and this may contribute to muscular complications of cholesterol lowering therapy.
- Individual variation within the reference range
- Variable bioavailability of supplements
- Low levels may be associated with a worse prognosis in vascular disease.
- Clinical research into the significance of CoQ in vascular disease.

Measuring plasma CoQ

Canterbury Health Laboratories

- HPLC with electrochemical detection
- CV < 5%
- Lithium heparin plasma
- Sample protected from light
- Storage at -80°C



Extraction of plasma CoQ

Canterbury Health Laboratories

- CoQ is more soluble in lipids than in lower alcohols such as ethanol.

- Ethanol and methanol are not miscible with with triacylglycerols.

- Two-phase extraction, evaporation, and redissolution in ethanol gives low yields, much of the CoQ remaining with the undissolved plasma lipid.

- Good recoveries can be obtained by extraction into 1-propanol with about 7 vol propanol to 1 vol sample.

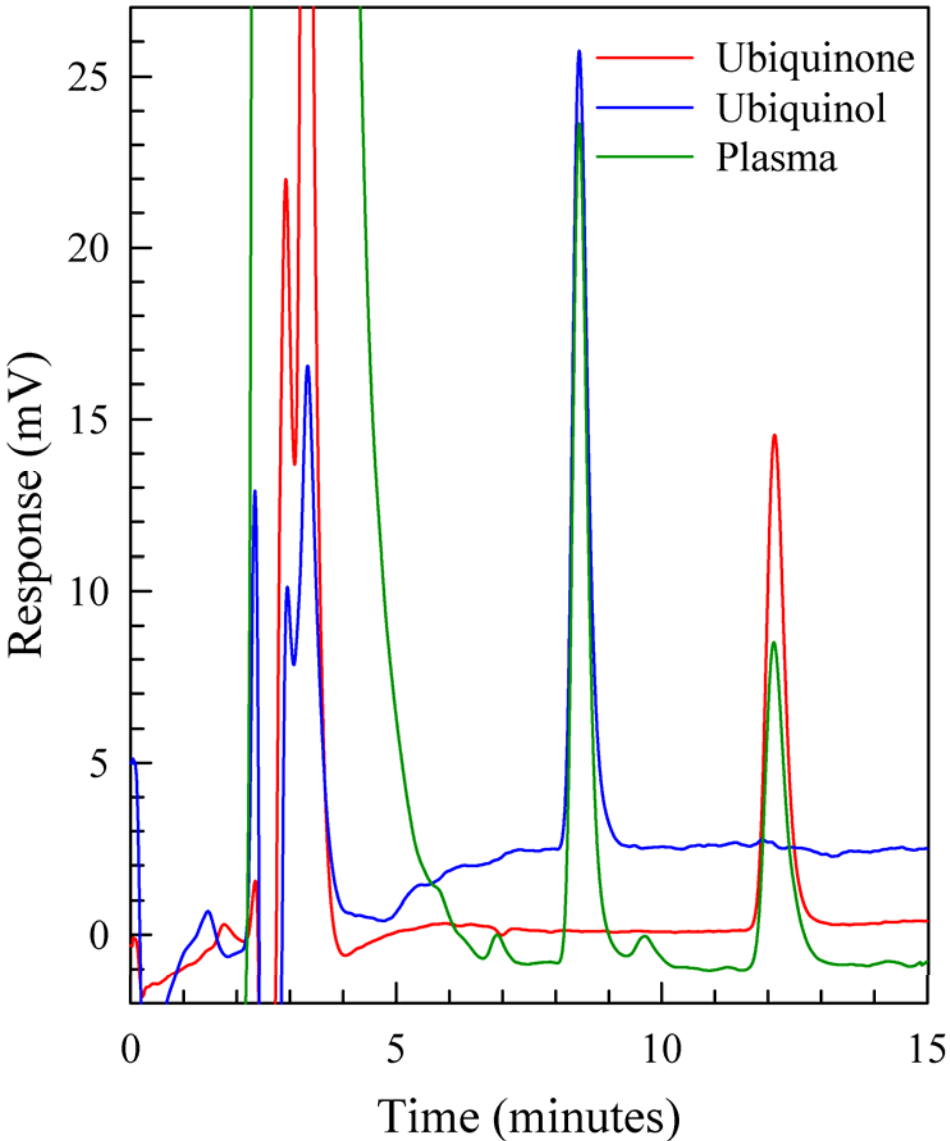
Extraction of plasma CoQ

Canterbury Health Laboratories

- CoQ is not strongly absorbed by normal phase silica, so practical mobile phases (for normal phase chromatography) contain very little polar modifier.
- As a result it is difficult to extract CoQ into an injection solvent that does not have a lot more eluting power than the mobile phase.
- Most methods use reverse phase columns with highly non-polar mobile phases.

Chromatography of plasma CoQ

Canterbury Health Laboratories

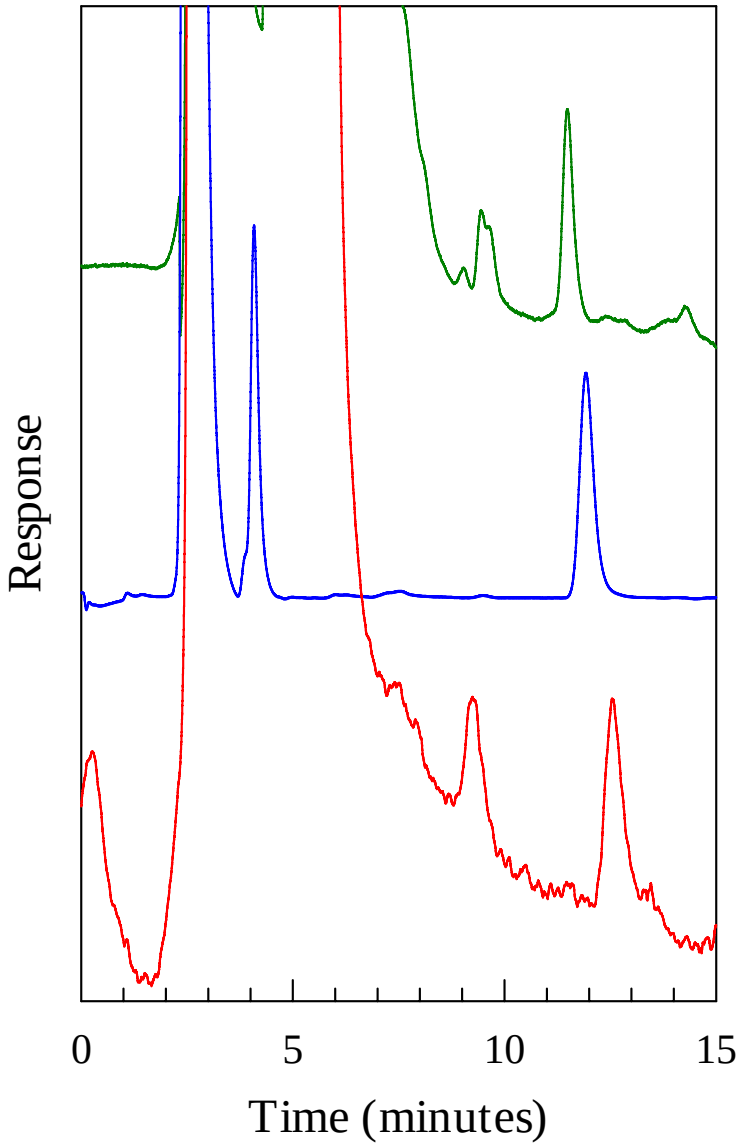


Reduced coenzyme Q_{10} (ubiquinol) is more hydrophilic than the quinone and elutes earlier from a reversed phase column.

Clean separations are easily achieved.

Detection of plasma CoQ

Canterbury Health Laboratories



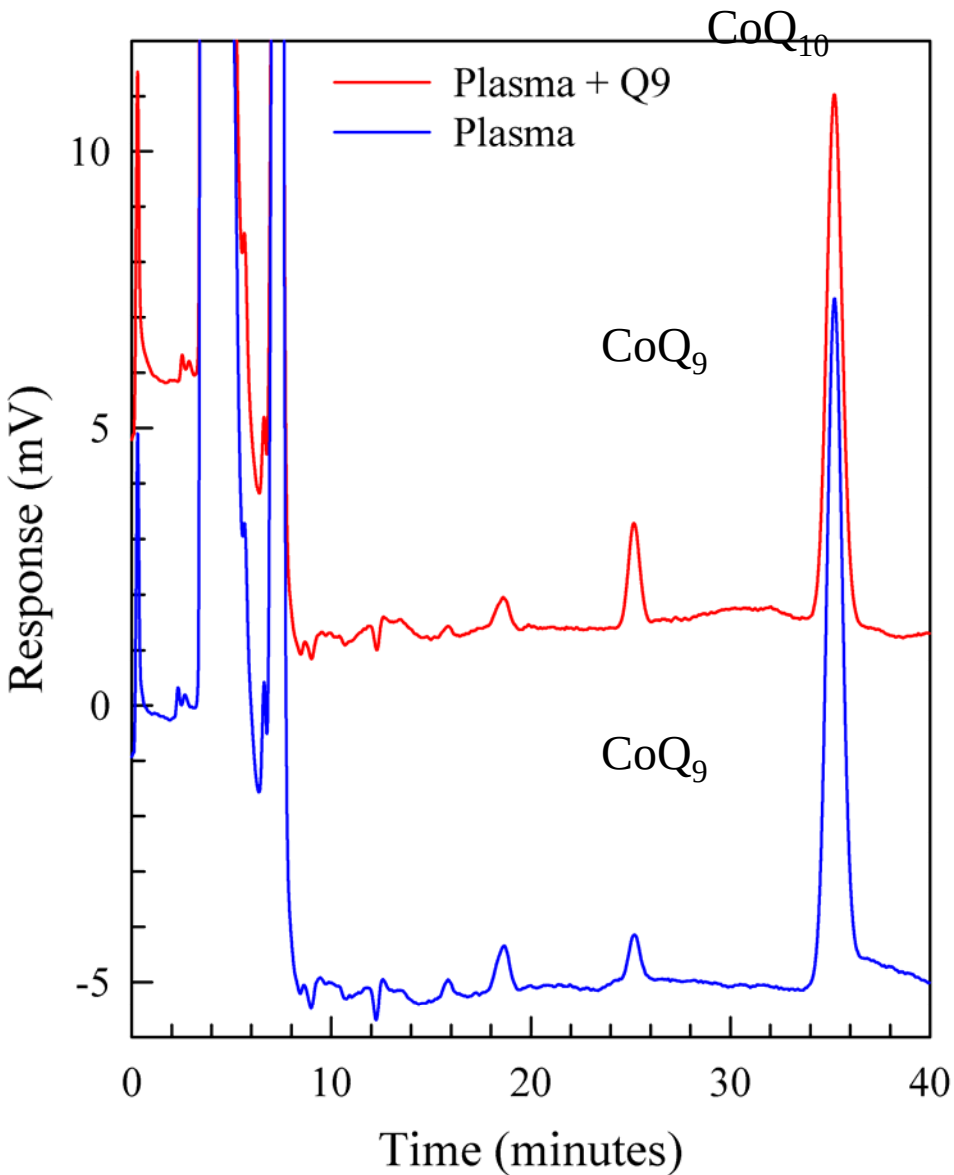
UV detection (275 nm)

Electrochemical detection

Fluorescence detection

Analysis of plasma CoQ

Canterbury Health Laboratories



- Coenzyme Q_9 was found in all human plasma samples studied.
- Illustration of separation on a C30 column.
- Separation (and co-chromatography with added standard) was confirmed on three different columns.

Internal standards for plasma CoQ

Canterbury Health Laboratories

CoQ_9 has been widely used as an internal standard. But it is present in normal human plasma.

The ethyl analogue of coenzyme Q_{10} has been used: this needs to be synthesized.

However, with propanol extraction the efficiency is close to 100% so internal standards are not really necessary.

Precision, recovery and assay range

Canterbury Health Laboratories

	Between-Run %CV	Within-Run %CV	Recovery (%)	Concentration Range (µmol/L)
Ultraviolet detection assay	3.2	2.4	93 - 103	0.24 - 0.98
Electrochemical detection assay	3.3	3.2	98 - 102	0.15 - 2.76

No effect of anticoagulants on ultraviolet assay, CoQ in EDTA plasma samples on average 4.4 ± 2.9% lower than in lithium heparin and serum samples (c.f. total cholesterol)

Reference Interval

Canterbury Health Laboratories

Ninety five percent reference intervals

	n	95% interfractile reference interval
Total CoQ_{10}	205	0.46 – 1.78 µmol/L
Total CoQ_{10} – Males	90	0.45 – 2.05 µmol/L [a]
Total CoQ_{10} - Females	115	0.46 – 1.71 µmol/L [a]
Total CoQ_{10} – Age 18 – 44 years	105	0.43 – 1.61 µmol/L
Total CoQ_{10} – Age 45 – 83 years	100	0.57 – 1.95 µmol/L

[a] stratification not required according to Harris and Boyde criteria

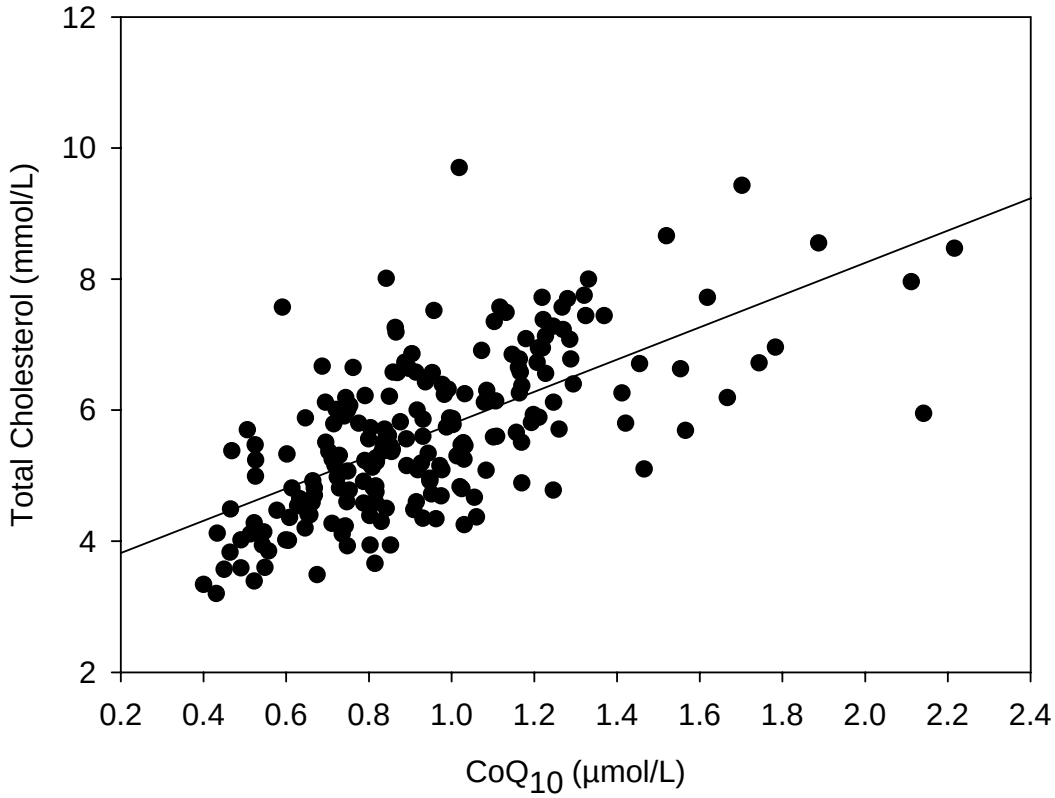
No significant difference between fasted (N = 115) and non-fasted (N = 90)

Measured CoQ, total cholesterol, and direct LDL cholesterol.

Correlation of CoQ with plasma lipids

Canterbury Health Laboratories

Significant correlation between CoQ and total (r = +0.651) and LDL-Cholesterol (r = +0.600). Both p < 0.001)



Biological variation

Canterbury Health Laboratories

- Healthy young male volunteers (N = 10)
- 7 fasting baseline measurements at least a week apart, over 2 month period
- Measured CoQ, LDL-Cholesterol, total cholesterol, and HDL-Cholesterol, all had healthy lipid levels

Inter- and Intra- individual variation

Canterbury Health Laboratories

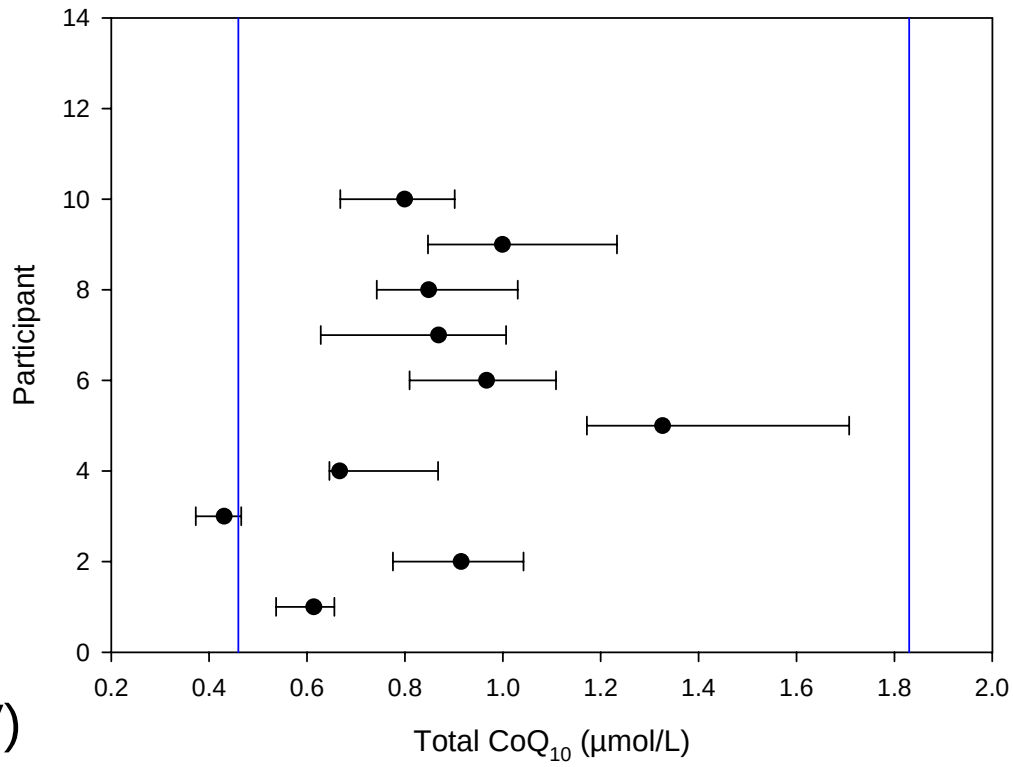
	Intra-individual %CV	Inter-individual %CV
Total CoQ	12	29
CoQ to LDL-C ratio	15	26
CoQ to TC ratio	14	18

- CoQ is tightly distributed around a homeostatic set point

Inter- and Intra- individual variation

Canterbury Health Laboratories

- Index of individuality (II)
- Low index (<0.6) - values for an individual span a small part of the reference interval
- High index (>1.4) - values for an individual cover most of the reference interval
- For total CoQ10, II = 0.42



- Reference change value (RCV)

$$RCV = 2^{1/2} \times Z \times (CV_a^2 + CV_i^2)^{1/2}$$

- For total plasma CoQ_{10}, RCV = 35% (for a 95% significant change)
- Example: Total CoQ_{10} concentration = 1 µmol/L

⇒ 95% significant change is below or over 0.65, or 1.35 µmol/L

Bioavailability of CoQ supplements

Canterbury Health Laboratories

- Seven different CoQ supplement brands
  - Blackmores - CoQ dissolved in **oil + surfactant**
  - Solgar - **dry** powder
  - Q-Gel - CoQ dissolved in **oil + surfactant**
  - Thompsons - CoQ dissolved in **oil**
  - Good Health - **dry** powder, chewable tablets
  - Radiance - CoQ dissolved in **oil + surfactants**
  - Kordels - CoQ in dissolved in **oils**

Bioavailability of CoQ supplements
Supplement adherence

Canterbury Health Laboratories

	mg of CoQ_{10} per Capsule/Tablet		
Brand	Claimed	Measured (mean ±SD)	Yield Recovery (%)
Q-Gel	30	41 ± 1.3	137
Radiance	50	63 ± 2.1	125
Blackmores	50	60 ± 4.1	121
Solgar	30	39 ± 4.4	130
Kordel's	75	95 ± 5.5	127
Thompson's	30	36 ± 1.9	121
Good Health	30	30 ± 2.0	100

(n = 6 tablets or capsules)

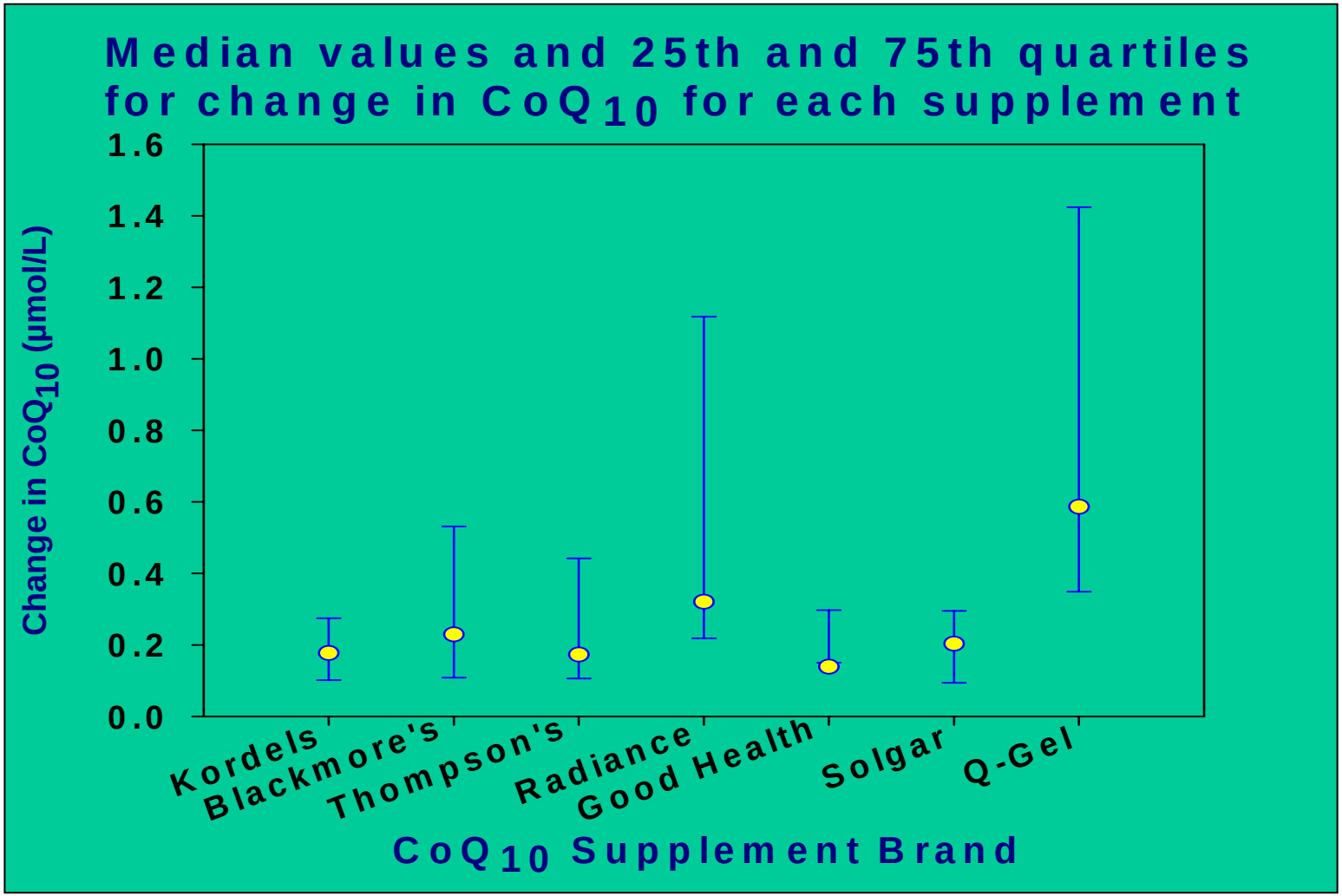
Bioavailability of CoQ supplements

Canterbury Health Laboratories

- Healthy young males (N = 10)
- Given single nominal 150 mg dose of each supplement brand, at least a week apart
- Blood samples taken at baseline, and six hours after ingestion of supplement
- Standardised breakfast and lunch provided (total CoQ content of diet approximately 315 µg)

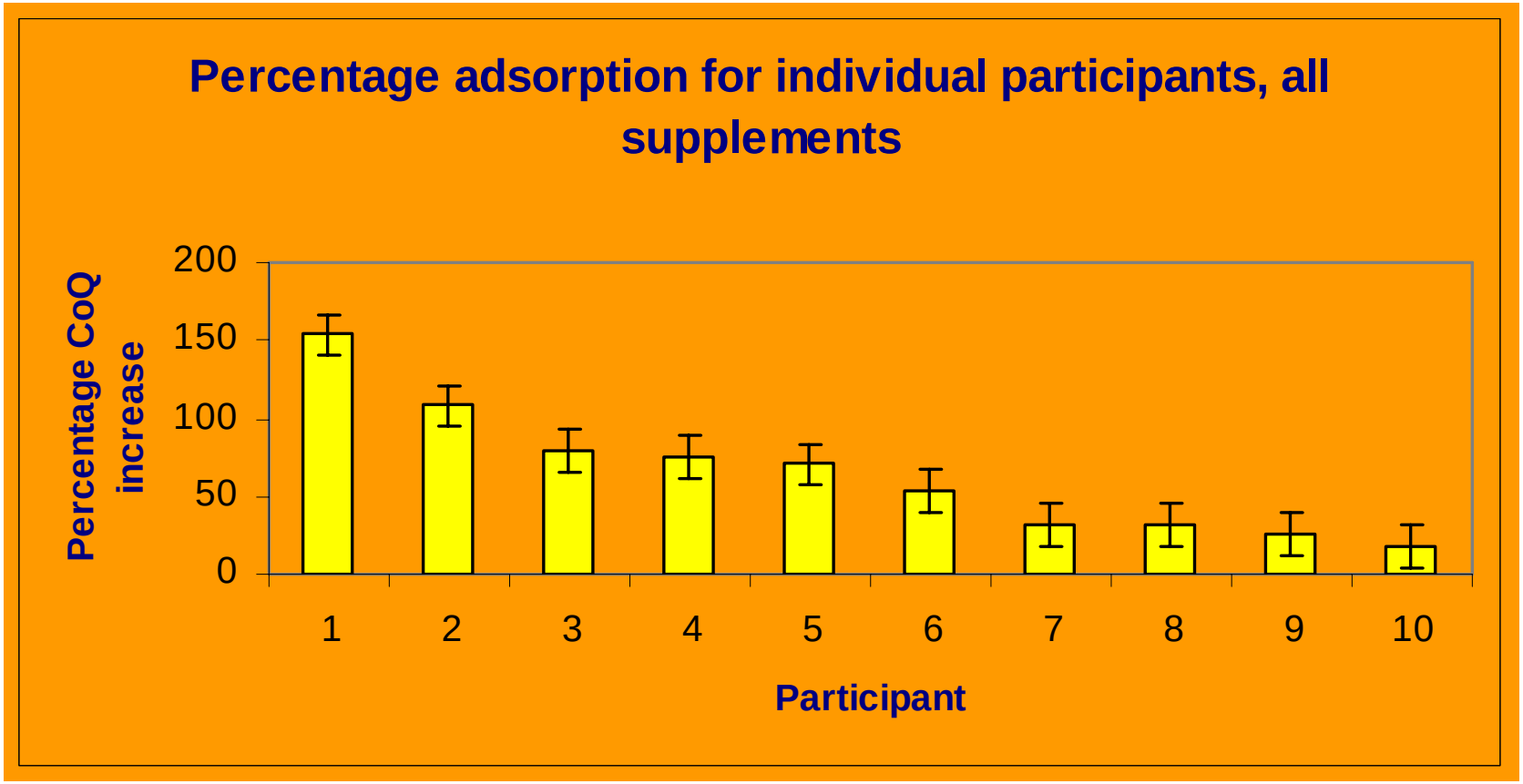
Bioavailability of CoQ supplements

Canterbury Health Laboratories



Bioavailability of CoQ_{10} supplements

Canterbury Health Laboratories



Primary CoQ deficiency

Canterbury Health Laboratories

- A rare, apparently autosomal recessive disorder with a clinical spectrum that encompasses three major phenotypes:
  - A myopathic form, characterised by exercise intolerance, mitochondrial myopathy, myoglobinuria, epilepsy, and ataxia
  - A generalised infantile variant with severe encephalopathy and renal disease
  - An ataxic form, dominated by ataxia, seizures, and cerebellar atrophy

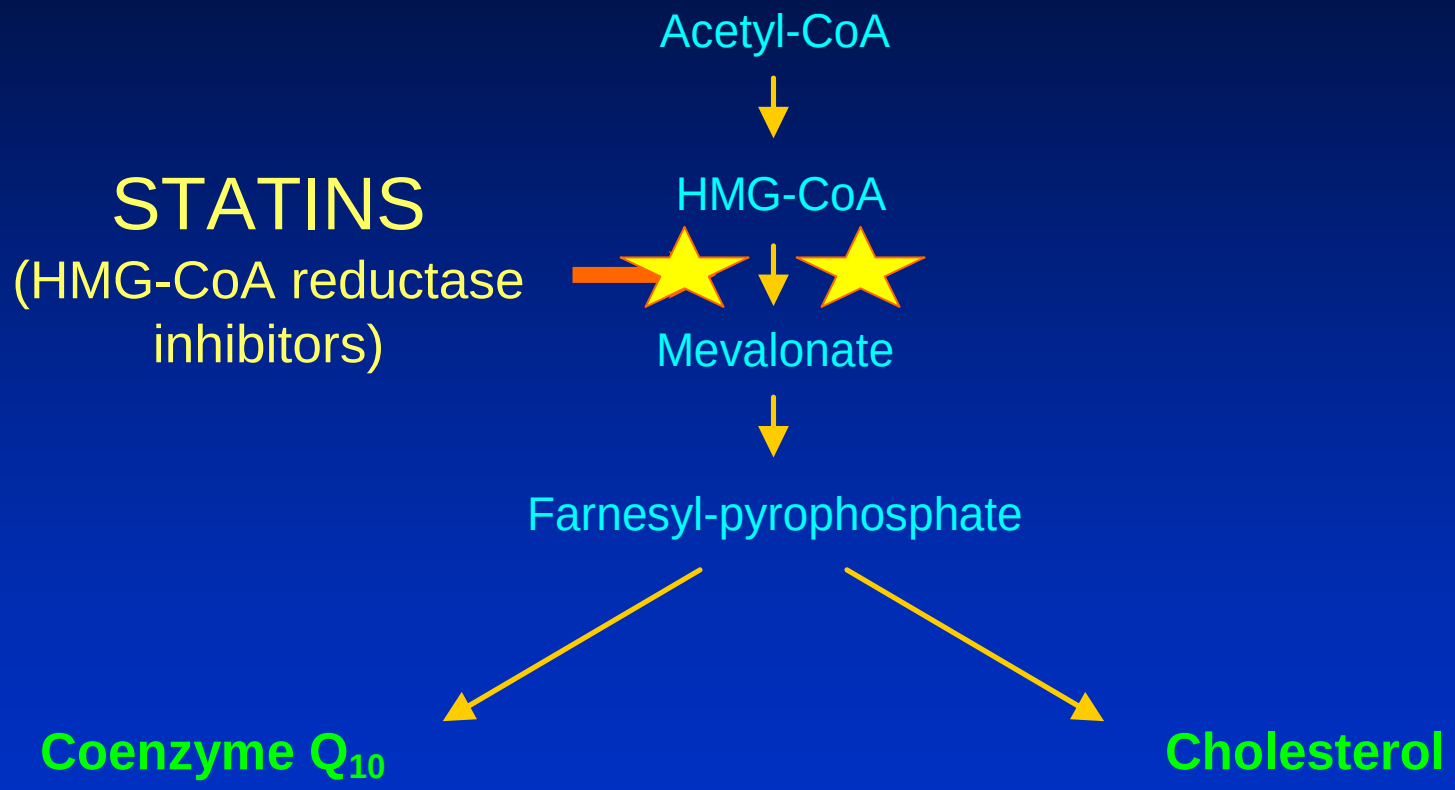
CoQ and disease

Canterbury Health Laboratories

Deficiency is relevant in

- Statin-induced myalgia
- Congestive Heart Failure
- Hypertension
- Parkinsons disease
- Alzheimers disease
- Chronic fatigue
- Infertility
- Cancer
- Diabetes

Statins Inhibit HMG-CoA Reductase

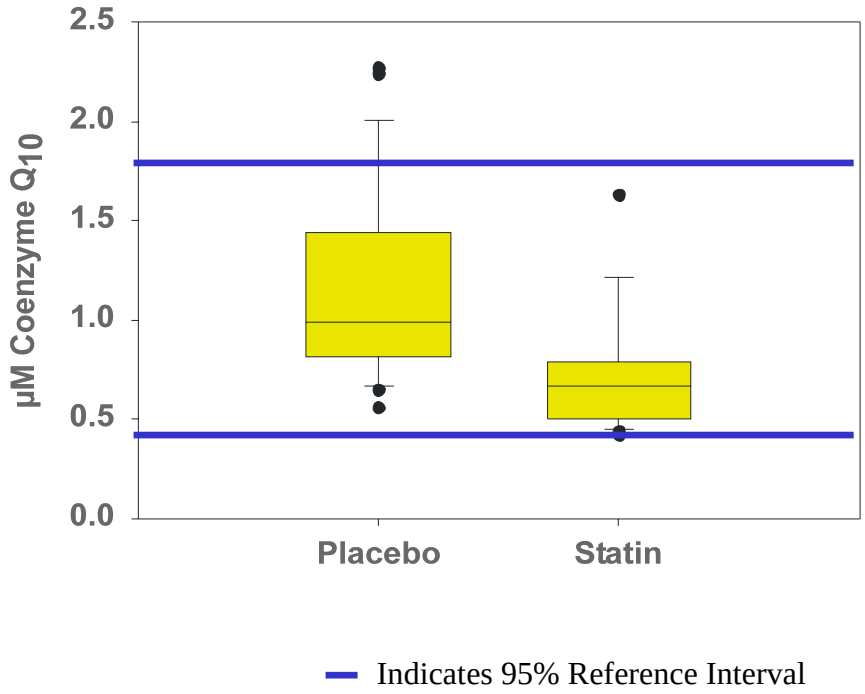


The mevalonate pathway links cholesterol and CoQ synthesis

Statin- induced CoQ deficiency

Canterbury Health Laboratories

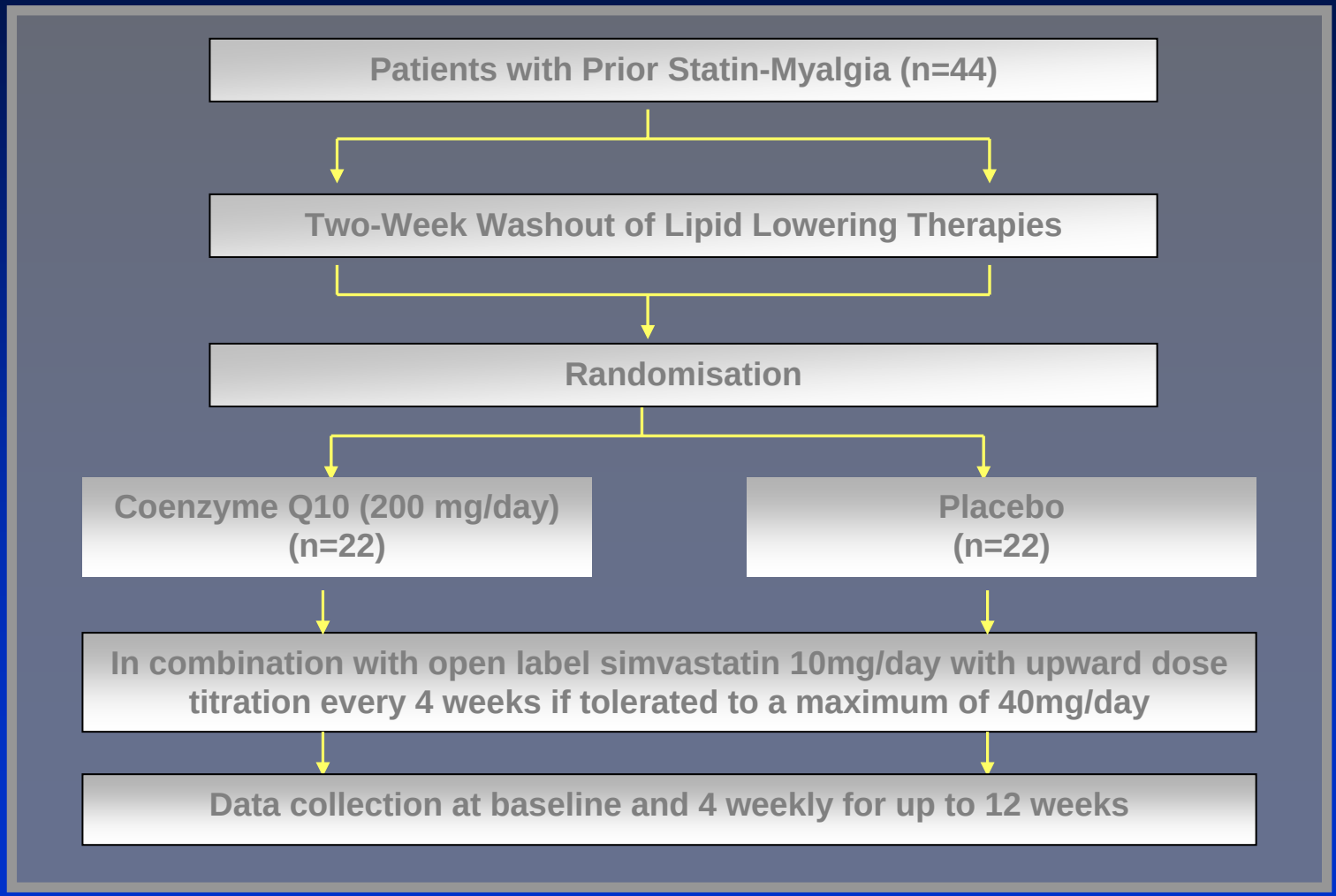
- 24 CHF patients
- Randomised placebo-controlled study
- 40 mg Atorvastatin for six weeks
- 33% CoQ reduction ($p < 0.001$)



Statin-Induced Myopathy

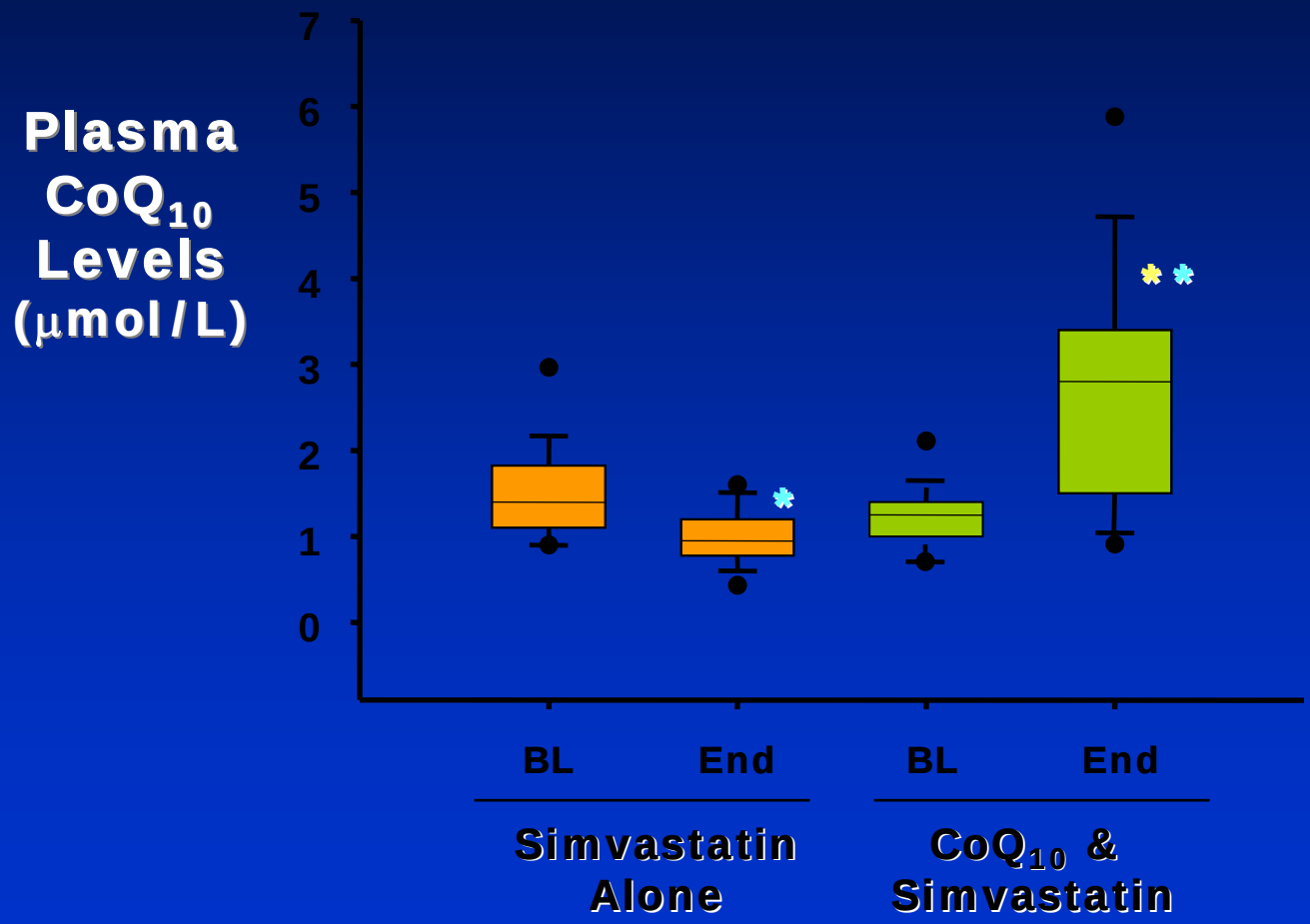
- Most frequently reported side effect of statin therapy
- Local data → 13% myalgia on statins
  Scott et al. N Z Med J. 1991; 104: 493-5
- Often necessitates reduction in statin dose or cessation of treatment

Effect of CoQ Supplementation on Simvastatin-Induced Myalgia



Young et al. Am J Cardiol (accepted 2007)

Changes in Plasma CoQ Levels



* p<0.001 for comparison between regimes

* p<0.001 for within group changes from baseline

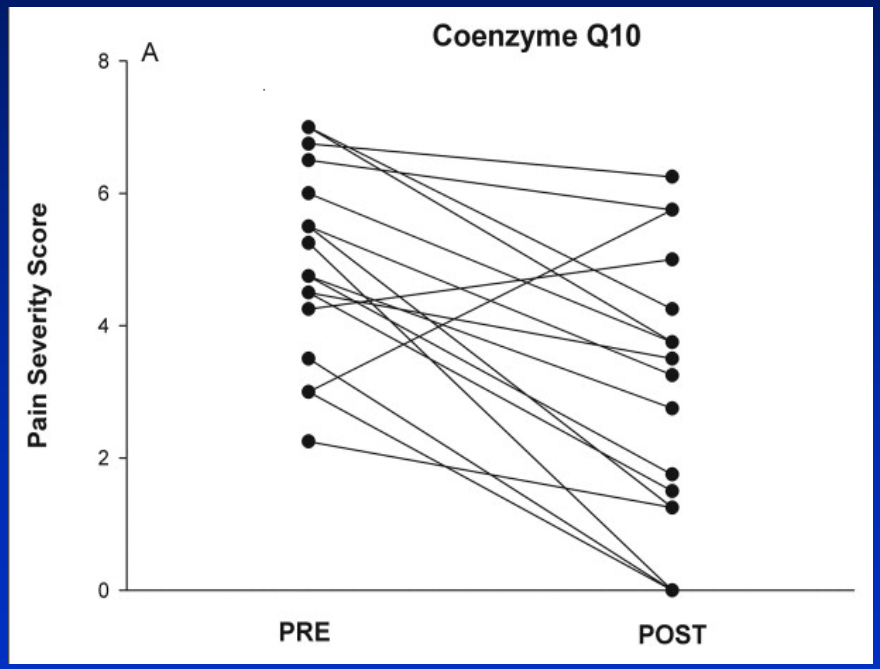
Statin Dose Tolerated at 12 Weeks

Dose tolerated	Simvastatin Alone (n=22)	CoQ_{10} & Simvastatin (n=22)
40mg/day	13 (59%)	16 (73%)
20mg/day	3 (14%)	0 (0%)
10mg/day	2 (9%)	0 (0%)
0	4 (18%)	6 (27%)

Values are counts (percentages). χ^2 test – no significant differences

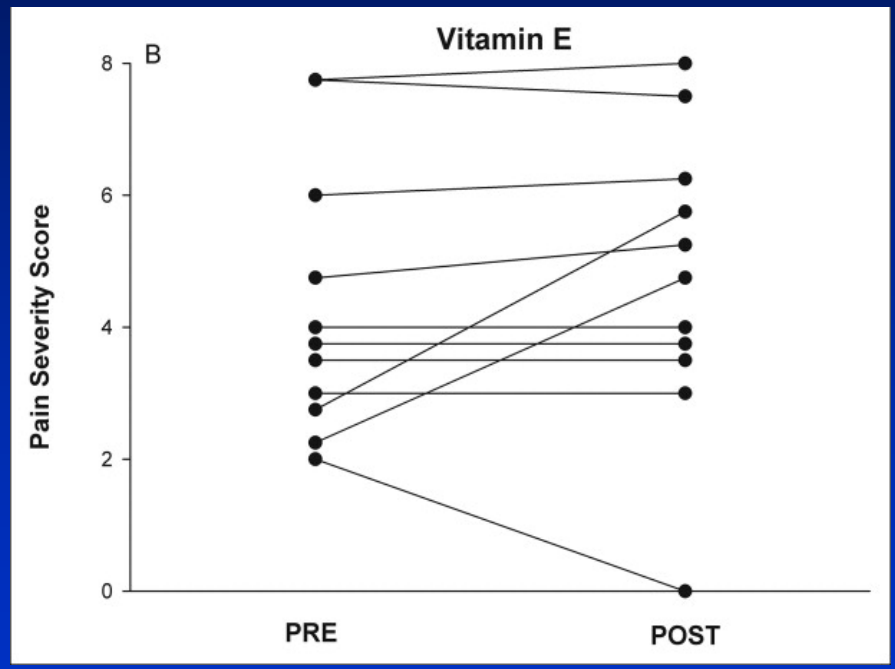
Changes in Pain Severity Scores

Coenzyme Q_{10} (n=18)



40% reduction in pain score (p<0.001)

Vitamin E (n=14)



No change in pain score (NS)

Caso et al. Am J Cardiol 2007; 99: 1409-12

CHF Background: CoQ_{10} and CHF

Canterbury Health Laboratories

- Reduced levels of CoQ_{10} have been reported in plasma and myocardium of patients with chronic heart failure [1]

- Q-SYMBIO (Coenzyme Q_{10} as adjunctive treatment of chronic heart failure. A randomised double-blind multicenter trial with focus on SYMptoms, Biomarker status (BNP) and long-term Outcome) [2]

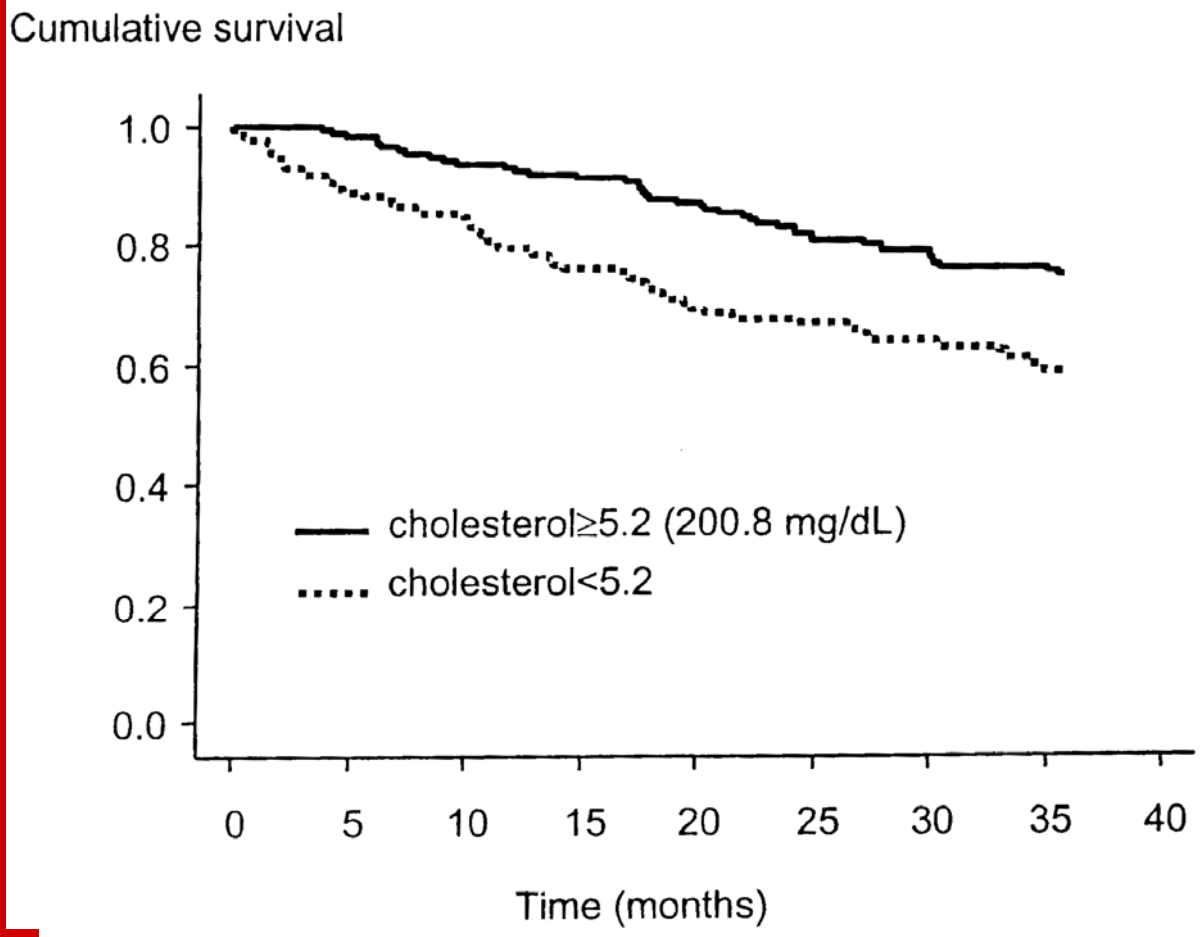
- An International study, expected completion late 2010
- 550 patients in NYHA classes III-IV in randomised parallel groups to receive 300 mg CoQ10 daily vs placebo on the top of stable, current treatment
- 6-minute walk tests and NTproBNP status assessment
- Long-term follow-up to evaluate the effects on morbidity (unplanned cardiovascular hospitalisations) and mortality as a composite endpoint in patients with severe heart failure receiving optimal medical therapy

[1] Folkers, K. et al. PNAS 1985. 82:901-4

[2] Mortensen, S.A. et al. Biofactors 2003. 18:79-9-89

CHF Background: Total cholesterol

Canterbury Health Laboratories



- 5.2 mmol/L was the best predictor of mortality by ROC-curve analysis
- Log-rank p=0.0011 for the difference between groups
- n=303

n=126 below 5.2 mmol/L
n=177 above 5.2 mmol/L

- 36-month survival 59% in below 5.2 mmol/L and 75% in above 5.2 mmol/L group.

Rauchhaus, M. et al., J Am Coll Cardiol 2003. 42:1933-40

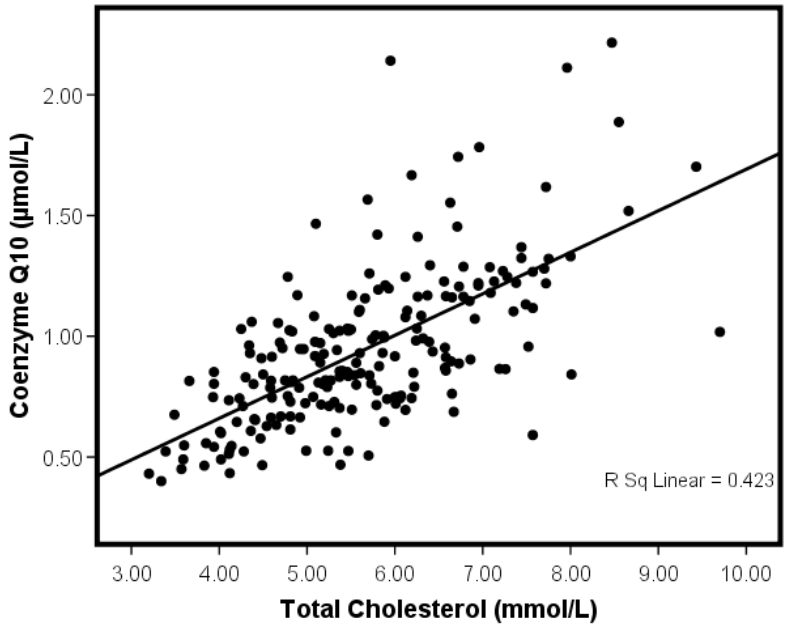
CHF Background: CoQ_{10} therapy

Canterbury Health Laboratories

- ATP generation is critical for cardiac function
- In Japan, adjunctive therapy with CoQ_{10} has been an accepted medication for CHF since the 1970's
- Also in parts of Europe and Russia CoQ_{10} is considered a part of standard therapy for congestive heart failure patients
- Plasma CoQ_{10} correlates with plasma lipids

n=205

(Healthy New Zealand Reference Range)



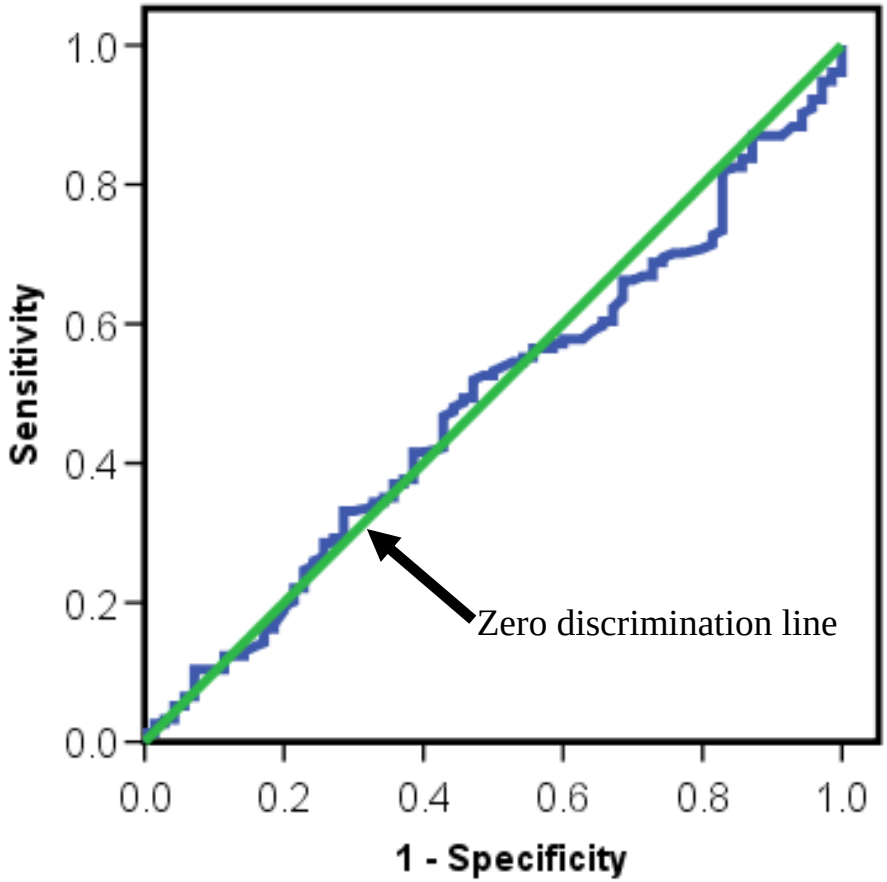
Study Objective and Methods

Canterbury Health Laboratories

- To investigate the association of plasma CoQ_{10} concentrations and mortality in a chronic heart failure population
- Randomised, controlled and blinded study
- Patients recruited 2-4 weeks post-discharge from index hospital admission with CHF
- N=236
- Median (range) follow-up was 2.69 (0.12 - 5.75) years

ROC curve – Total Cholesterol

Canterbury Health Laboratories

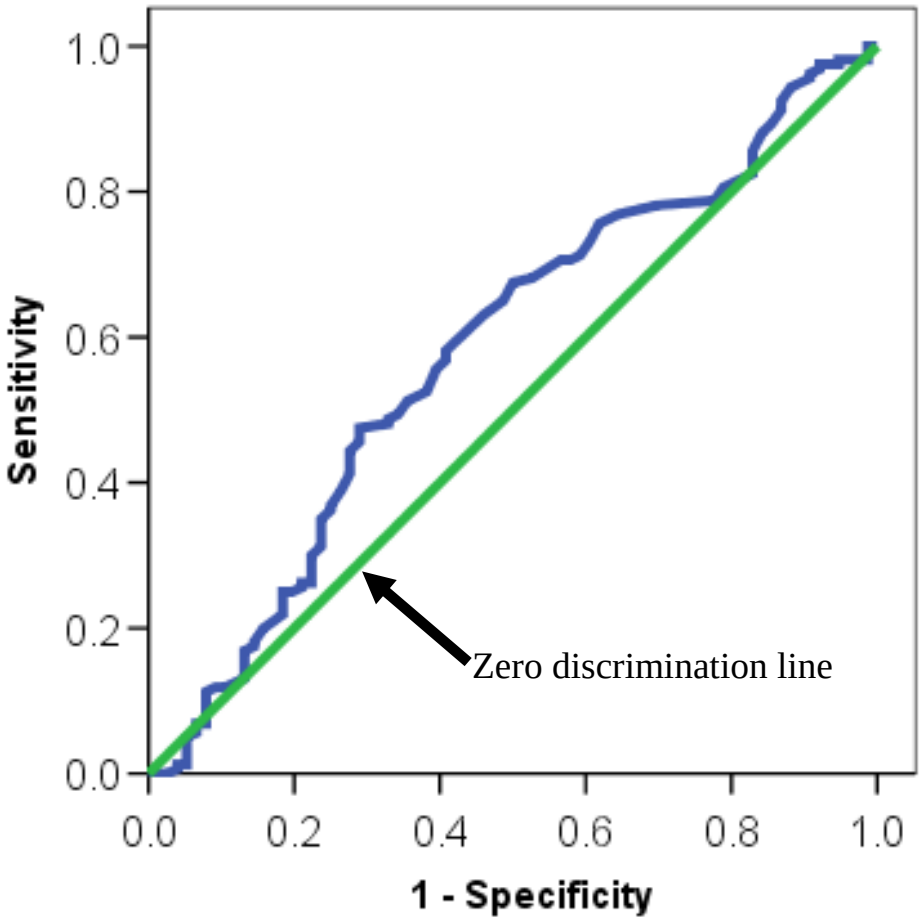


- AUC (± SE) = 0.486 ± 0.041
- P* = 0.740
- Optimal cut-off cannot be determined due to non-significant ROC-curve

(* p-value is for difference from a random effect)

ROC curve – CoQ_{10}

Canterbury Health Laboratories

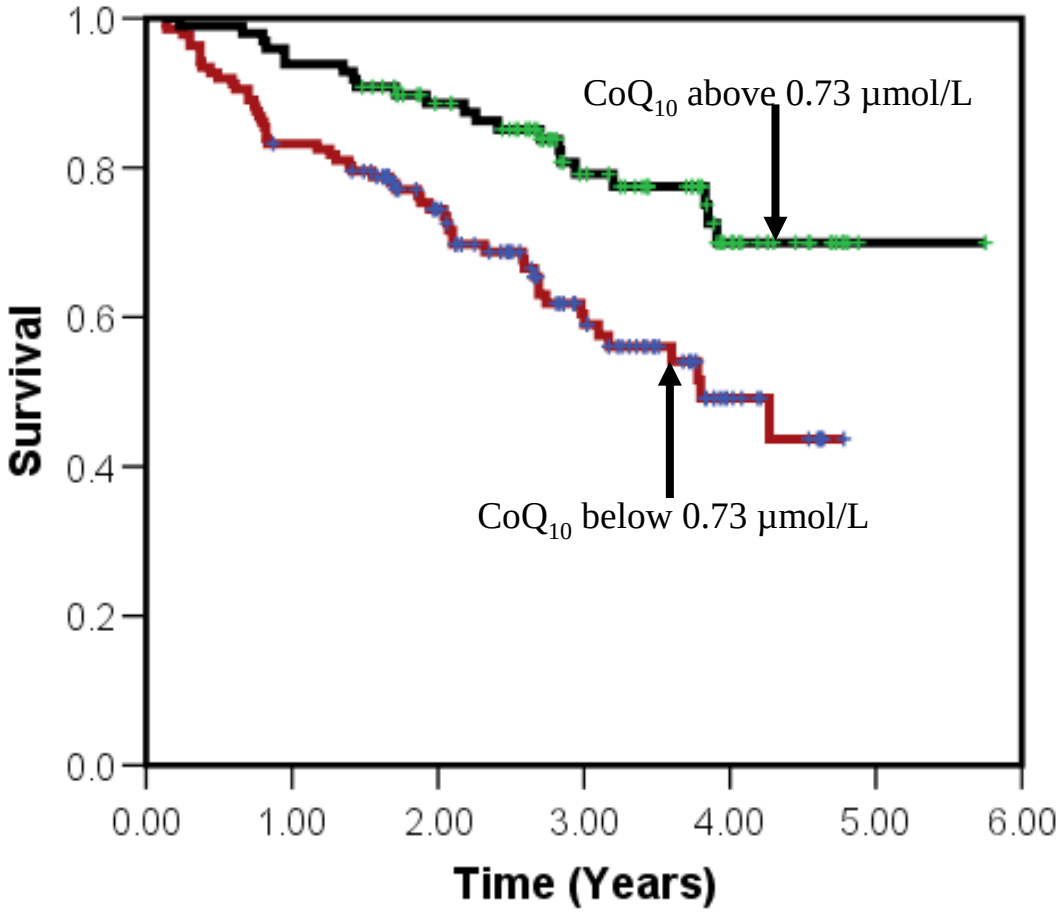


- $P^{*} = 0.041$
- AUC (± SE) = 0.582 ± 0.040
- Optimal cut-off = 0.73 µmol/L

(* p-value is for difference from a random effect)

CoQ_{10} Kaplan-Meier

Canterbury Health Laboratories

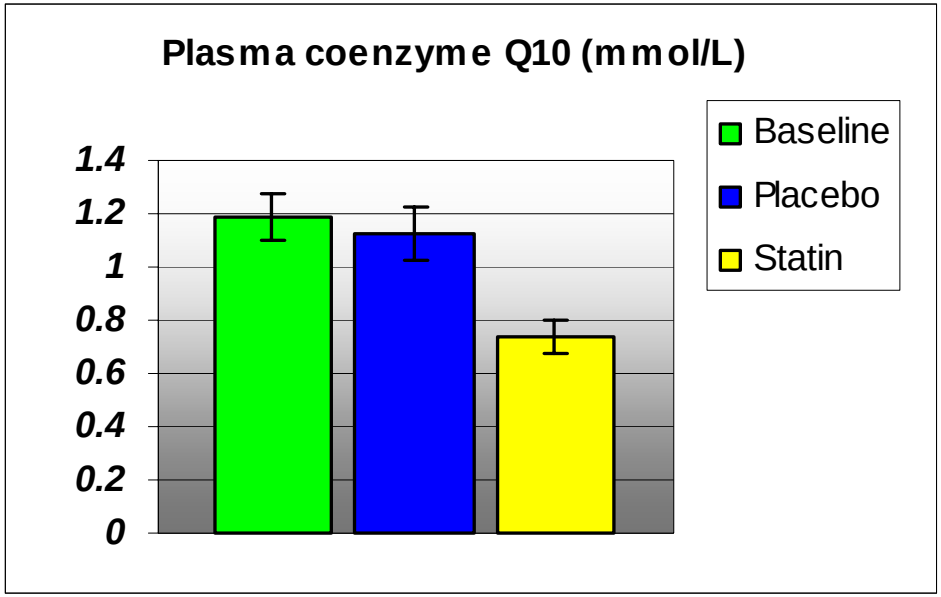


- Survival with CoQ_{10} above and below the ROC-curve determined best predictive value of mortality.
- Log-rank $p<0.001$ for the difference between groups

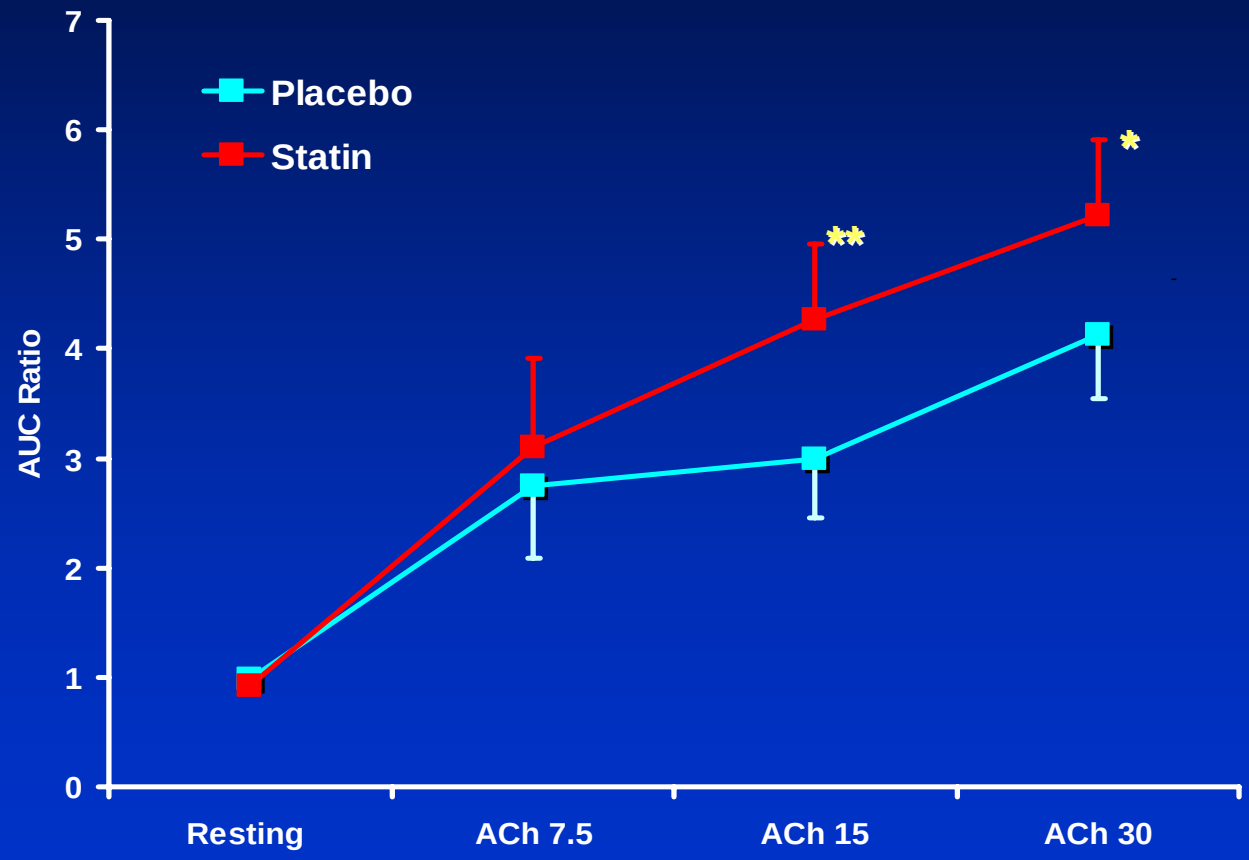
CoQ10 and endothelial function: Study Design

Canterbury Health Laboratories

- Double- blind, placebo controlled cross over 6-wk study.
- 24 patients with CHF [NYHA II or III, LVEF < 40%].

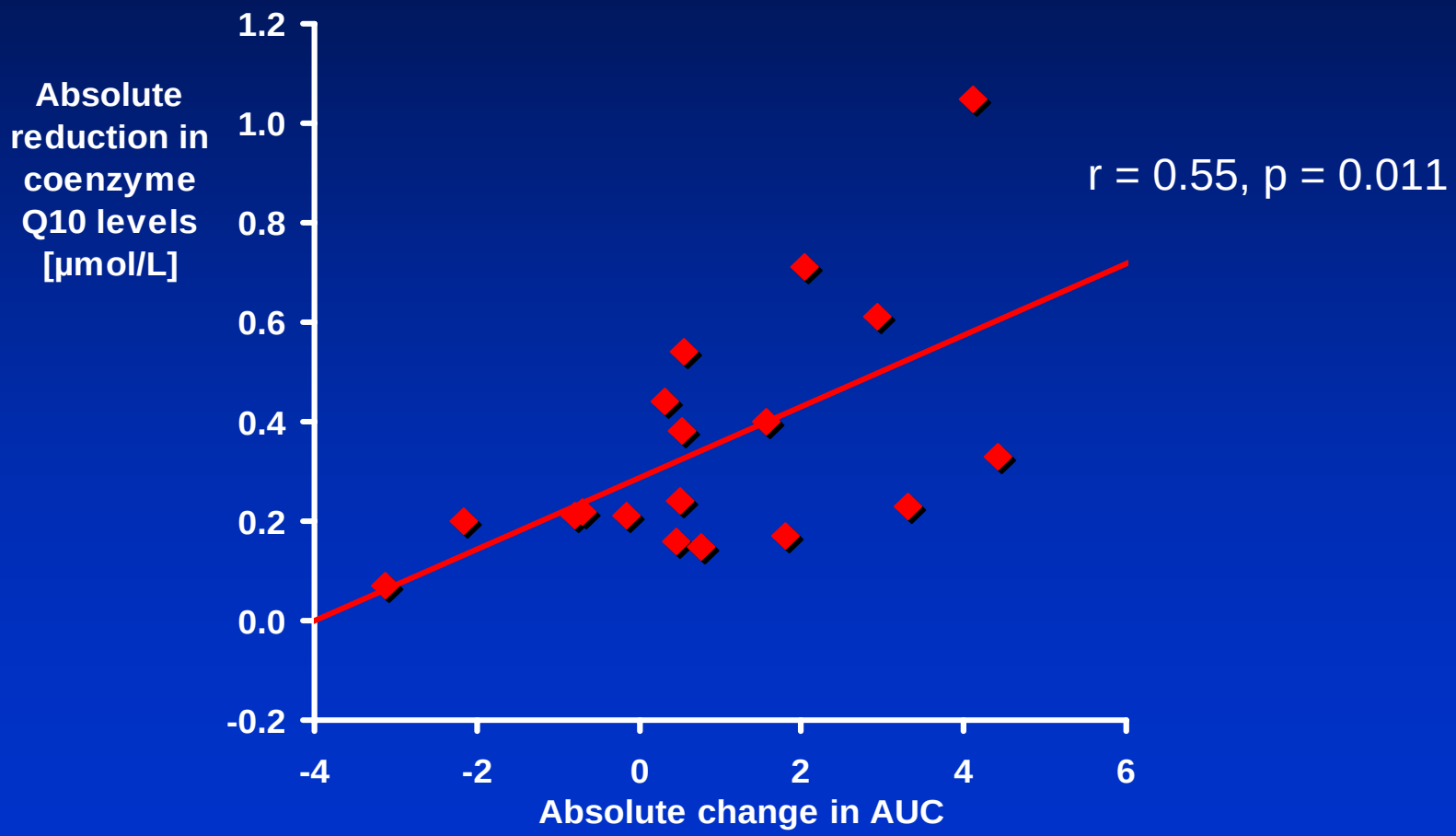


Statin therapy improved endothelial function in CHF



AUC = Area under the curve, ACh = Acetylcholine (7.5 - 30 µg/min), * p<0.05, ** p<0.01

Plasma Coenzyme Q_{10} Reduction vs Improvement in Endothelial Function Post Statin Therapy



* during ACh infusion at 30 ug/min

CoQ and Diabetes

- Plasma CoQ reduced in diabetes.
- CoQ shown to improve HBA1c, glucose levels, reduce insulin levels
- CoQ also reduced blood pressure.
- CoQ improved vascular function (FMD), but not microcirculation.
- Combination CoQ and Fibrate treatment improved microcirculatory function.

CoQ and Hypertension

- Recent meta-analyses – 12 trials
- Decrease in systolic blood pressure: **11-17mmHg**
- Decrease in diastolic blood pressure: **8-10mmHg**
- Antioxidant mechanism
- CoQ maybe useful adjunct in resistant hypertension and patients with side effects.

Conclusions

- CoQ is clinically relevant to a number of diseases
- Supplementation may be indicated in heart failure, statin induced myalgia, diabetes and hypertension
- Monitoring is appropriate during supplementation

LIPID AND DIABETES RESEARCH GROUP

Collaborators:

CANTERBURY HEALTH LABORATORIES

UNIVERSITY OF OTAGO