CHAPTER 7

Effects of dietary intake of Coenzyme Q_{10} on healthy individuals

Introduction

The benefits or otherwise of antioxidants to the health of the individual, either by an adequate dietary intake or by dietary supplementation, has been a key issue in the health care industry for several decades. Epidemiological observations have suggested that antioxidants may play a protective role in the prevention and/or protection of disorders that are associated with ROS. To date, none of the large-scale, randomised studies have unequivocally supported the epidemiological evidence. In fact, one study demonstrated an increased risk of lung cancer in male smokers supplemented with antioxidants (β-carotene) compared with male smokers who were not supplemented (Omenn *et al.*, 1996; Goodman *et al.*, 2004).

Antioxidants are also thought to have a role in slowing the ageing process. According to the *free radical theory of ageing,* the accumulation of oxidative damage caused by ROS is largely responsible for the general decline of health we experience with age (Beckman and Ames, 1998). Accrual of cellular ROS is highly damaging to DNA, proteins and lipids initiating cellular impairment. During the normal ageing process, metabolic and antioxidant functions decline further leading to the deterioration of tissue and organs as well as late-onset degenerative diseases (Linnane *et al.*, 1998; Knight, 2000; Holbrook and Ikeyama, 2002).

The role of Coenzyme Q

Ubiquinone (or coenzyme Q) is ubiquitous in nature and belongs to a group of substances known as quinones, hence the name ubiquinone. Ubiquinone is a lipid-soluble component of the electron-transport chain found in both prokaryotic and eukaryotic cells.

Ubiquinone (2,3-dimethoxy-5-methylbenzoquinone) consists of a quinone ring attached to a side chain consisting of a number of monounsaturated trans-isoprenoid units and is structurally similar to vitamin K [Fig 7.1]. In eukaryotes, it is found in the mitochondria and also in other cellular structures such as the endoplasmic reticulum, the Golgi apparatus, lysosomes, peroxisomes and plasma membranes (Søballe and Poole, 1999). This distribution suggests ubiquinone has roles additional to that of a redox carrier.

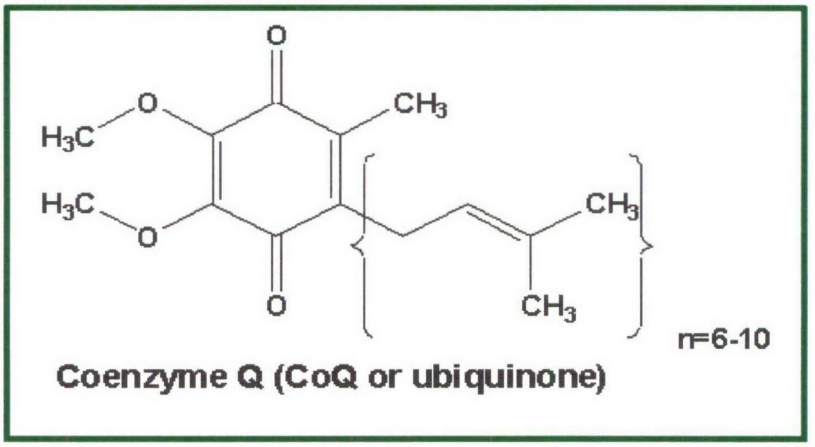


Figure 7.1. Chemical structure of coenzyme Q where n = 6 – 10

In eukaryotic cells, ubiquinone is located in the hydrophobic region of the inner membrane of the mitochondrion, and can move freely within this area by way of diffusion. The enzyme NADH-ubiquinone oxidoreductase reduces ubiquinone to ubiquinol, thus ubiquinone acts as a redox electron carrier. The transport of electrons down the chain then continues with ubiquinol passing on an electron to a protein complex (complex II) known as cytochrome reductase [Fig 7.2]. Reactive oxygen species, including hydrogen peroxide (H_2O_2), superoxide radicals ($\bullet O_2^-$), hydroxyl radicals (OH•), peroxyl radicals (ROO•) and singlet oxygen are generated as by-products of normal cellular metabolism. Autoxidation of the electron transport carriers in mitochondria is a known source of superoxide anion generation and, as such, can elicit damage to proteins, lipids and DNA (Halliwell and Gutteridge, 1999; Davidson and Schiestl, 2001). Preventing the formation of ROS in mitochondrial electron transport is, therefore, a clear strategy for reducing cellular oxidative stress.

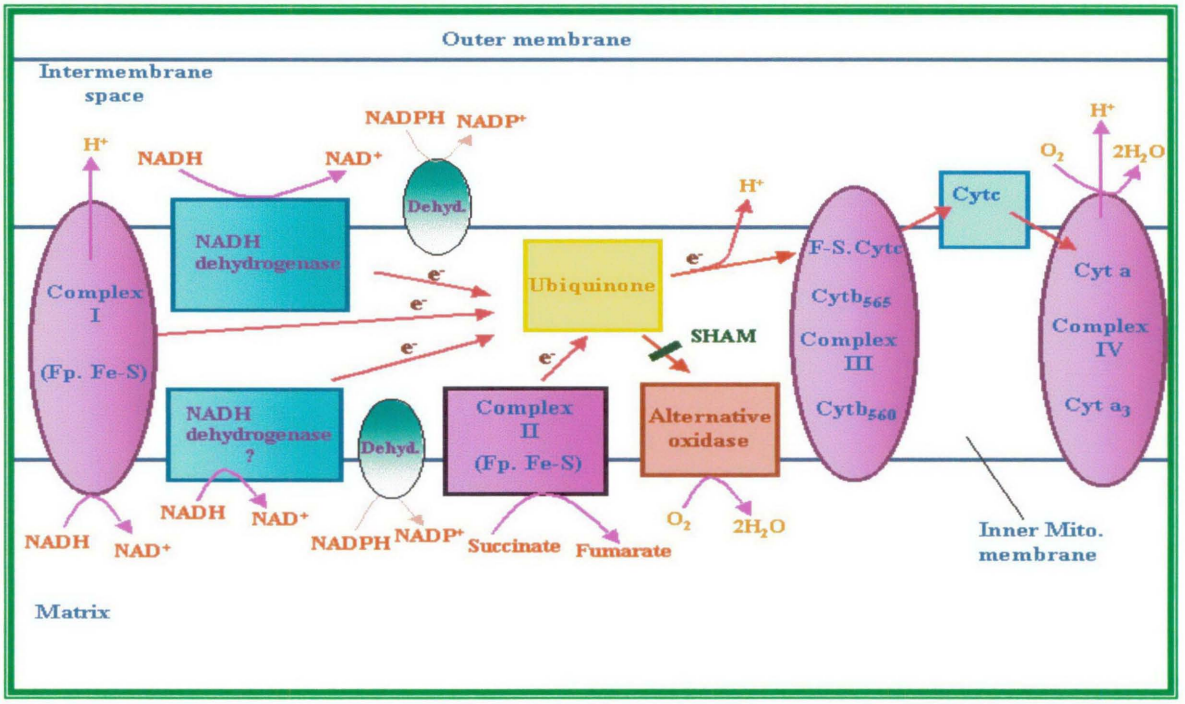


Figure 7.2. The location of ubiquinone in the inner mitochondrial membrane (www.uky.edu/~dhild/ biochem/10/lect.html)

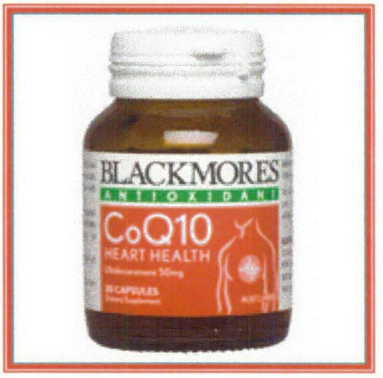
The ratio of ubiquinone to ubiquinol has been used as a marker of oxidative stress in both bacteria (Søballe and Poole, 1999; 2000; Dunlap *et al.*, 2002) and human plasma (Beyer *et al.*, 1996; Yamamoto and Yamashita, 1997). NAD(P)H:quinone oxidoreductase (NQR, DT-Diaphorase) is a two-electron quinone reductase that forms and maintains coenzyme Q in the reduced state (Beyer *et al.*, 1996). Impaired capacity to maintain adequate reduced levels of ubiquinol has been linked to various disease states such as Parkinson's disease, hepatitis, liver cirrhosis, hyperlipidemia, cardiovascular disease (Kontush *et al.*, 1997; Soja and Mortensen, 1997; Yamamoto and Yamashita, 1999; Gotz *et al.*, 2000) and various types of cancer such as leukemia (Larson *et al.*, 1999; Wiemels *et al.*, 1999; Smith *et al.*, 2001; Krajinovic *et al.*, 2002b), colorectal cancer (Traver *et al.*, 1992; Harth *et al.*, 2000), and breast cancer (Marin *et al.*, 1997; Marks-Hull *et al.*, 1997). Ubiquinone has been shown to have antioxidant properties by increasing resistance to oxidation in various systems such as lymphocytes, plasma membranes and lipoproteins (Kontush *et al.*, 1997; Tomasetti *et al.*, 1999; Kaikkonen *et al.*, 2002) by inhibiting lipid peroxidation and scavenging free radicals. Ubiquinol is capable of regenerating vitamin E from its oxidised form, the alpha-tocopheroxyl radical, although this process is normally facilitated by vitamin C. This interaction with vitamin E is thought to

provide protection of low-density lipoprotein (LDL) from oxidative damage. Reduced levels of low density lipoprotein (LDL) by oxidative damage is correlated with increased risk of ischemic heart disease (Stocker *et al.*, 1991; Bowry *et al.*, 1995). When present in adequate concentrations, ubiquinol reduces the rate of LDL peroxidation. Upon ubiquinol depletion, LDL peroxidation increases leading to atherosclerosis (Stocker *et al.*, 1991). The reported deficiencies in the levels of ubiquinone in the myocardial tissue of patients with chronic heart disease (CHD) initiated many studies into the clinical outcome of cardiac patients treated with CoQ_{10}. Results have indicated improved mitochondrial bioenergetics thus leading to the use of CoQ_{10} as treatment for those suffering or recovering from CHD (Langsjoen *et al.*, 1985; Singh *et al.*, 1998).

There have been very few studies evaluating the effect that CoQ_{10} may have on the immune system. One pilot study (Folkers *et al.*, 1991) reported a significant increase in blood IgG and T4-lymphocytes following CoQ_{10} supplementation in fourteen healthy individuals, leading to speculation that CoQ_{10} may be of clinical benefit in the treatment of HIV due to deficiencies in CoQ_{10} (Folkers *et al.*, 1988). It has been postulated that the immune response may be modified in monocytes, lymphocytes and granulocytes following enrichment with CoQ_{10} (Turunen *et al.*, 2002). However, there have been no reports of controlled clinical trials evaluating the effectiveness of CoQ_{10} on the immune response. The present study (6 males, 6 females) was conducted to evaluate the effects of CoQ_{10} supplementation in healthy subjects with respect to the potential protective effects against oxidative stress. In addition, stress protein synthesis in human lymphocytes was measured as previous studies from this laboratory reported that hsp70, which is associated with the immune system, was stimulated in lymphocytes isolated from individuals on an antioxidant supplement (Peng *et al.*, 2000; Howard *et al.*, 2002).

Methods

Study population



Six males and six females, aged between 34 and 61 years, participated in the study and were recruited through the University of New England. Participants were in good general health, non-smokers and were not taking any prescribed or non-prescribed medications. Recruitment and human research ethics approval took 4 months. Participants took two 50 mg capsules per day for fourteen days, one in the morning and one in the evening, containing the active ingredient ubidecarenone (CoQ_{10}, Blackmores Ltd., Sydney, Australia). Participants gave written informed consent with the study design. Consent forms and methodology were approved by the Human Research Ethics Committee of the University of New England, Armidale [HREC approval number HE01/202]. Trained personnel at New England Pathology, Armidale, collected approximately 30 ml of peripheral blood into lithium heparin Vacuette® blood collection tubes (Becton Dickinson, Sydney) following an overnight fasting period on day one, day fourteen and again following a washout period of a further fourteen days.

Lymphocyte extraction

Lymphocytes were separated from whole blood by density gradient centrifugation using Ficoll-Paque (Amersham Biosciences), according to the manufacturer's instructions. Twenty ml of whole blood was gently mixed with 15 ml RPMI media (methionine free). Ten ml of Ficoll-Paque was underlaid, taking care not to disturb the interface. Samples were centrifuged at 400 x *g* for 30 min without braking or acceleration. Lymphocytes were collected into fresh tubes and mixed with enough RPMI media to give a final volume of 25 ml, followed by centrifugation at 1350 x *g* for 5 min. The supernatant was discarded and the lymphocytes washed again using 10 ml of RPMI media. The supernatant was again discarded and the lymphocytes resuspended in RPMI media (2 ml) along with 10% foetal bovine serum.

^{35}S-methionine labelling of proteins

Protein synthesis in control and heat shocked lymphocytes were examined by incorporation of ^{35}S-methionine into protein. 150 µCi/ml of ^{35}S-methionine (70% L-methionine [^{35}S] and 15% L-cysteine [^{35}S], specific activity 1175 Ci/mmol) was added to the lymphocyte suspension at 37°C.

Stress response

Lymphocytes were heat shocked by placing lymphocyte suspensions in a water bath at 42.5°C for 1 hr and then allowed to recover for 3 hr at 37°C. Control samples remained in the 37°C water bath for the duration of the experiment.

Protein extraction

Following heat shock, samples were washed in 800 µl RPMI to remove excess label, centrifuged and the supernatant removed. Fifty µl of protein extraction buffer was added and samples frozen overnight at –20°C. Samples were thawed on ice the following day with intermittent vortexing, centrifuged at 13000 x *g* for 3 min and the supernatant transferred into a fresh microfuge tube. Samples were stored at –80°C until required.

Protein assay

For lymphocytes, 5 µl of protein sample was added to 495 µl ddH_2O and 500 µl Pierce Coomassie protein assay reagent. The absorbancies of samples and bovine serum albumin (BSA) standards were measured against a reagent blank using a spectrophotometer at 595 nm. Protein content was calculated by interpolation from the constructed BSA standard curve.

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis method for separation of proteins is outlined in Chapter 2.

Autoradiography

Dried gels were exposed to Biomax MR film (Kodak) at –80°C for a time period of 8 to 14 days. Following incubation, films were brought back to room temperature and developed under infrared (1-2 min in Kodak X-ray developer, rinsed under running water 1 min, immersed in Kodak X-ray fixer 1 min, rinsed under running water 30 sec).

Western immunoblot

Protein samples (10 µg) and 10 µl Kaleidoscope protein standards (Bio-Rad) were separated using 10% iGels (Gradipore). Following electrophoresis, gels were submerged in continuous transfer buffer (39 mM glycine, 48 mM Tris, 20% (v/v) methanol, 0.0375% (w/v) SDS). Proteins were transferred to a nitrocellulose membrane (Hybond-C super) pre-equilibrated in transfer buffer using the Novablot electrophoresis transfer system (Pharmacia LKB, model 2117-250). A stack of nine filter papers, the nitrocellulose membrane, the iGel and another stack of nine filter papers were prepared (filter paper and membrane were all pre-equilibrated in transfer buffer). Transfer was carried out for 90 min at room temperature using a constant current of 120 mA. Upon completion, the membrane was submerged in blocking solution of PBS-T (100 mM NaCl, 80 mM Na_2HPO_4, 20 mM NaH_2PO_4, pH 7.4, 0.1% (v/v) Tween-20) incorporating 5% (w/v) skim milk powder.

Detection of antibody

Bound antibodies were detected using the ECL™ western immunoblotting detection kit (Amersham) following the manufacturer's instructions. Membranes were removed from the blocking solution the following day and quickly washed twice with PBS-T followed by a 15 min wash then 2 x 5 min washes, all in PBS-T. Membranes were then removed from solution and incubated with the primary antibody (StressGen Biotechnologies, Victoria, BC, Canada) (hsp 70; 1:2000 concentration) in PBS-T containing 5% skim milk powder in a sealed plastic bag with continuous rocking for 1 hr. Following incubation, the membrane was washed as described previously and re-incubated with the secondary antibody (anti-mouse) (1:1000 concentration) in PBS-T containing 5% skim milk powder following the same procedure as for the primary antibody. Following the second incubation period, the wash steps as previously described were repeated. Membranes were then incubated for a period of 1 min with the ECL™

detection reagents, drained and wrapped in cling-wrap. Membranes were then exposed to Hyperfilm-MP (Amersham) for varying periods of time, ranging from 30 sec to 5 min. Films were developed under infrared (1-2 min in Kodak X-ray developer, rinsed under running water 1 min, 1 min in Kodak X-ray fixer, rinsed under running water 30 sec).

Enzyme-linked immunosorbent assay [ELISA]

Detection and quantitation of hsp70 in protein extracts from lymphocytes was achieved using a sandwich immunoassay kit from StressGen Biotechnologies (StressXpress™ hsp70 ELISA Kit) according to the manufacturer's instructions. Inducible hsp70 is captured with a bound antibody (mouse monoclonal) followed by detection with a secondary antibody (rabbit polyclonal), thus completing the 'sandwich'. Measuring the amount of secondary antibody bound through the use of a stabilized tetramethylbenzidine substrate allowed quantification of the assay. Absorbance readings at 450 nm were read on a microplate reader. Calculations of hsp70 concentrations were made by constructing a standard curve using recombinant hsp70 standards and interpolating sample absorbances.

Erythrocyte haemolysis

Approximately 4 ml of whole blood was centrifuged at 600 x *g* for 10 min. The plasma and buffy coat were removed by aspiration. Plasma was stored at –80°C until required and the buffy coat was discarded. Erythrocytes were washed twice in 5 volumes of PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM K_2HPO_4, pH 7.4) by centrifugation at 600 x *g* for 10 min. Two hundred µl of erythrocytes were suspended in 800 µl of PBS (pre-warmed to 37°C) and 1 ml of 100 mM AAPH (2,2'-azobis-(2-amidinopropane) dihydrochloride) in PBS, pH 7.4. The suspension was then gently mixed and incubated at 37°C in a shaking water bath. At 30 min intervals, 50 µl aliquots of the cell suspension were diluted with 1.95 ml of 0.15 M NaCl, centrifuged at 1000 x *g* for 10 min and the absorbance measured at 540 nm. The percentage of haemolysis was calculated against the same volume of cell suspension diluted with 1.95 ml ddH_2O, representing 100% haemolysis.

Protein carbonyl assay

Protein carbonyls in plasma were determined as a measure of protein oxidation. Plasma samples were incubated with 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl for a period of 1 hr in the dark at room temperature with vortexing every 15 min. After incubation, protein was precipitated by the addition of 20% (w/v) TCA and allowed to stand for 15 min followed by vortexing and centrifugation at 13,000 x *g* for 5 min. Upon discarding the supernatant, the remaining pellet was washed (twice) in 1 ml of ethanol-ethyl acetate (1:1), pipette mixing to resuspend the pellet and then left to stand for 10 min followed by centrifugation at 13,000 x *g* for 3 min. This wash step removed any free DNPH and lipid contaminants from the sample. Proteins were then dissolved by the addition of 6 M guanidine in 20 mM KPO_4 (pH 2.3) and incubated for 1 hr at 37°C. Insoluble material was removed by centrifugation at 13,000 x *g* for 5 min. Blank samples were incubated with 600 µl 2 M HCl without DNPH at room temperature. Carbonyl content was calculated by spectrophotometric assay at 370 nm reading the DNPH treated sample against the blank (using the molar extinction coefficient of 22,000 M^{-1} cm^{-1} (Levine *et al.*, 1990; Reznick and Packer, 1994)).

Lipid peroxidation

Lipid peroxidation was estimated as thiobarbituric acid reactive substances (TBARS) and was measured as malondialdehyde (MDA). Determination of MDA in plasma was used to measure lipid peroxidation, before and after stress conditions. MDA forms with thiobarbituric acid a 1:2 adduct that can be measured by spectrophotometry. Plasma samples were mixed with 100 mM AAPH in PBS in 1.5 ml microfuge tubes and incubated for 3 hr in a 37°C water bath. Following incubation, samples were vortexed and transferred to a clean tube. One ml of TCA-TBA-HCl (15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid in 0.25 M HCl) solution was then added to all samples and thoroughly mixed by vortexing. Samples were heated in a boiling water bath for 15 min and then cooled to room temperature on ice. Following centrifugation at 3,000 x *g* for 3 min, the absorbance of the supernatant was determined spectrophotometrically at 535 nm against a TCA-TBA-HCl blank. The malondialdehyde (MDA) content of the sample was calculated using an extinction coefficient of 1.56×10^5 M^{-1}. cm^{-1} (Wills, 1969).

Total antioxidant status

The Calbiochem® Total Antioxidant Status Assay Kit was used to determine the total antioxidant status in plasma in accordance with the manufacturer's instructions. Reconstituted chromogen (1 ml) was mixed with 20 µl of plasma in a 2 ml cuvette. The initial absorbance (A_1) was measured at 600 nm with air as a blank. Two hundred µl of substrate solution was then added, mixed well and incubated in the spectrophotometer at 37°C for 3 min. The final absorbance (A_2) was then taken again at 600 nm. Antioxidants are measured by their ability to inhibit the oxidation of 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) to produce the radical cation $ABTS^{\cdot+}$ by the peroxidase metmyoglobin (Miller *et al.*, 1993). Under these conditions, antioxidants present will suppress the absorbance in a proportional manner to their concentration, as shown in Figure 7.3.

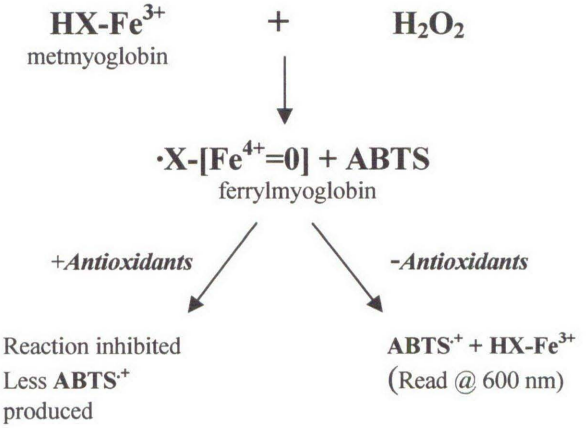


Figure 7.3. Principle of the Calbiochem Total Antioxidant Status Assay

Results

Erythrocyte haemolysis and CoQ_{10} supplementation

As illustrated in Figure 7.4, in the case of erythrocytes isolated from the male cohorts, the onset of haemolysis induced by AAPH was time-dependent with 50% haemolysis ($T_{50\%}$) at about 210 min. In the same individuals, and following dietary supplementation for two weeks with ubiquinone (2 x 50 mg tablets), the $T_{50\%}$ decreased to about 150 min. Following a two week wash-out period, the $T_{50\%}$ for erythrocyte haemolysis was retained at about 150 min. For the female cohorts [Fig 7.5] the $T_{50\%}$ for pre-supplementation was approximately 180 min and this decreased to 148 min following supplementation. Following the two week wash-out period, $T_{50\%}$ increased to around 185 min which was similar to the value obtained for pre-supplementation.

Heat shock protein synthesis in lymphocytes

Protein synthesis was measured in lymphocytes at 37°C (control) or at 42.5°C (heat-shock). Heat shock inducible proteins were identified by the incorporation of ^{35}S-methionine followed by separation by SDS-PAGE and analysis by autoradiography. These analyses clearly demonstrated the induction in lymphocytes of hsp70 and hsp90 synthesis following a heat shock. The results for both the female [Figs 7.6 – 7.8] and male [Figs 7.9 – 7.11] cohorts were very similar for lymphocytes from baseline, post-supplemented and wash-out samples. In order to quantitate any potential differences in induction of hsps, further analysis of hsp70 was conducted using western immunoblots. These results are illustrated in Figure 7.12 for baseline and post-supplemented samples with similar results for the wash-out samples (results not shown). Statistical analyses of the western immunoblots are summarised in Table 7.1. No significant differences were observed in any of the combinations of baseline versus post-supplementation, baseline versus wash-out or post-supplementation versus wash-out. The only exception was in the male cohort in which there was a statistical difference (p = 0.005) in hsp70 induction between the post-supplentation and the wash-out. In the latter samples there was a significant increase in hsp70 induction as observed by western immunoblots following a heat shock. In an attempt to resolve this observation, a commercially available ELISA kit for hsp70 was used. However, although the results from these arrays agreed in general with the western immunoblots, quantitative analyses of the data was judged to be of low value due to inconsistencies in the absorbancy readings of a number of the lymphocyte samples.

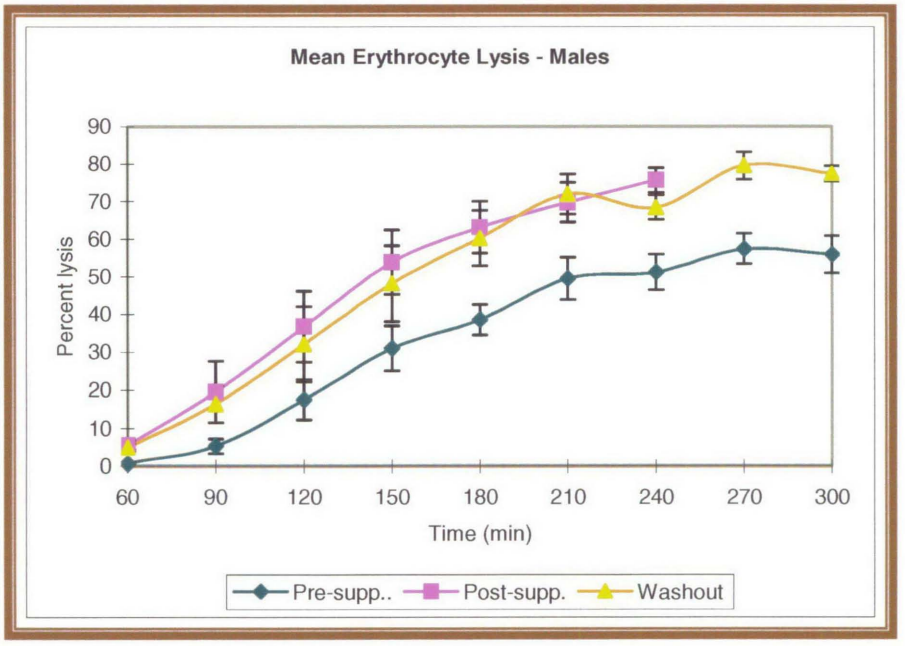


Figure 7.4. The effect of CoQ_{10} supplementation of AAPH induced erythrocyte haemolysis in males. n = 6.

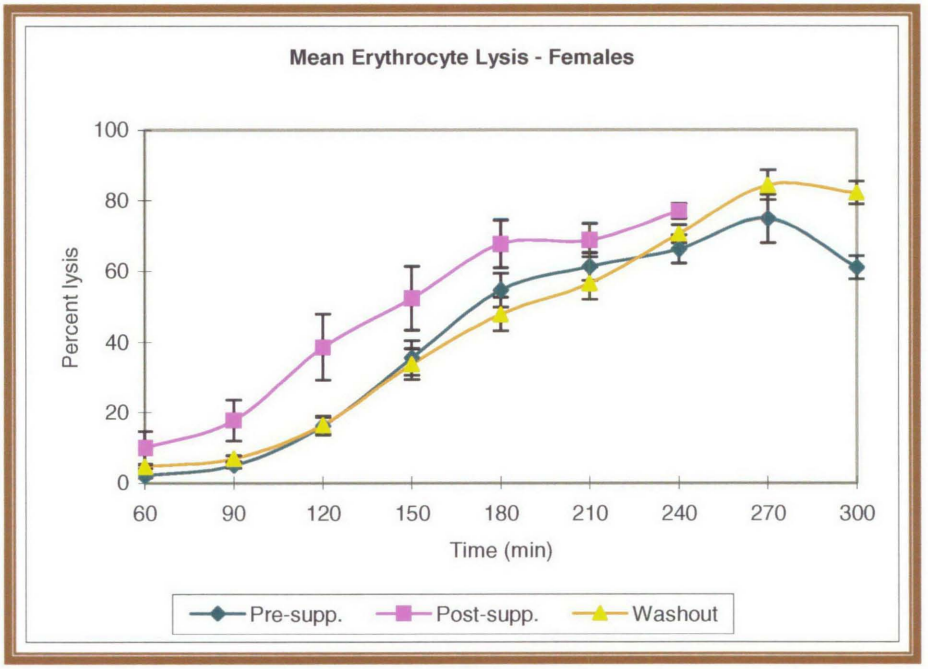


Figure 7.5. The effect of CoQ_{10} supplementation of AAPH induced erythrocyte haemolysis in females. n = 6.

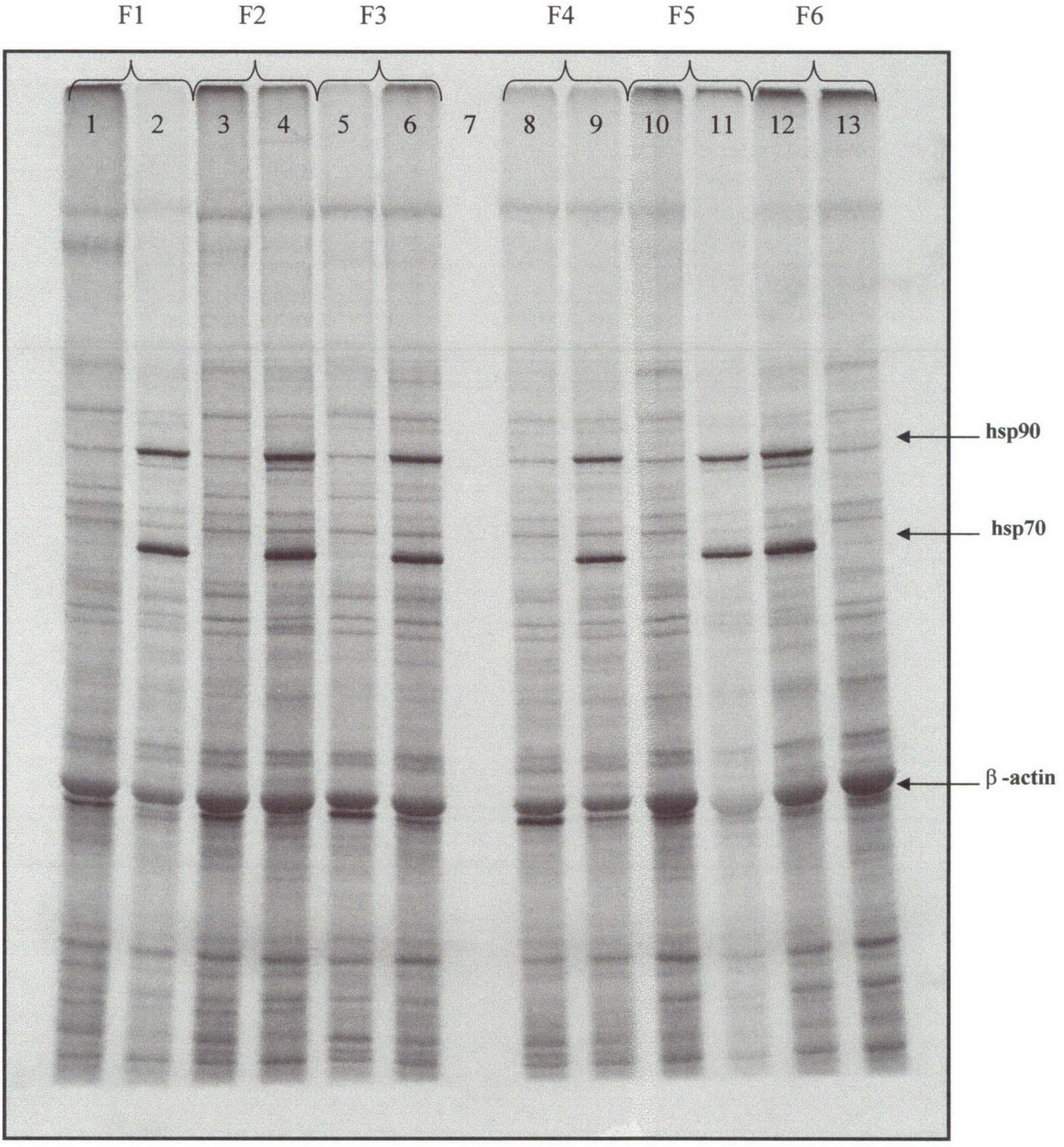


Figure 7.6. Autoradiogram of ^{35}S-methionine labelled lymphocyte proteins from the female cohort pre-supplementation with CoQ_{10}.

Lanes 1, 3, 5, 8, 10 and 13: control samples at 37°C throughout for participants F1, F2, F3, F4, F5 and F6 respectively.
Lanes 2, 4, 6, 9, 11 and 12: heat shocked samples at 42.5°C for 1 h followed by a 3 h recovery period at 37°C for participants F1, F2, F3, F4, F5 and F6 respectively.

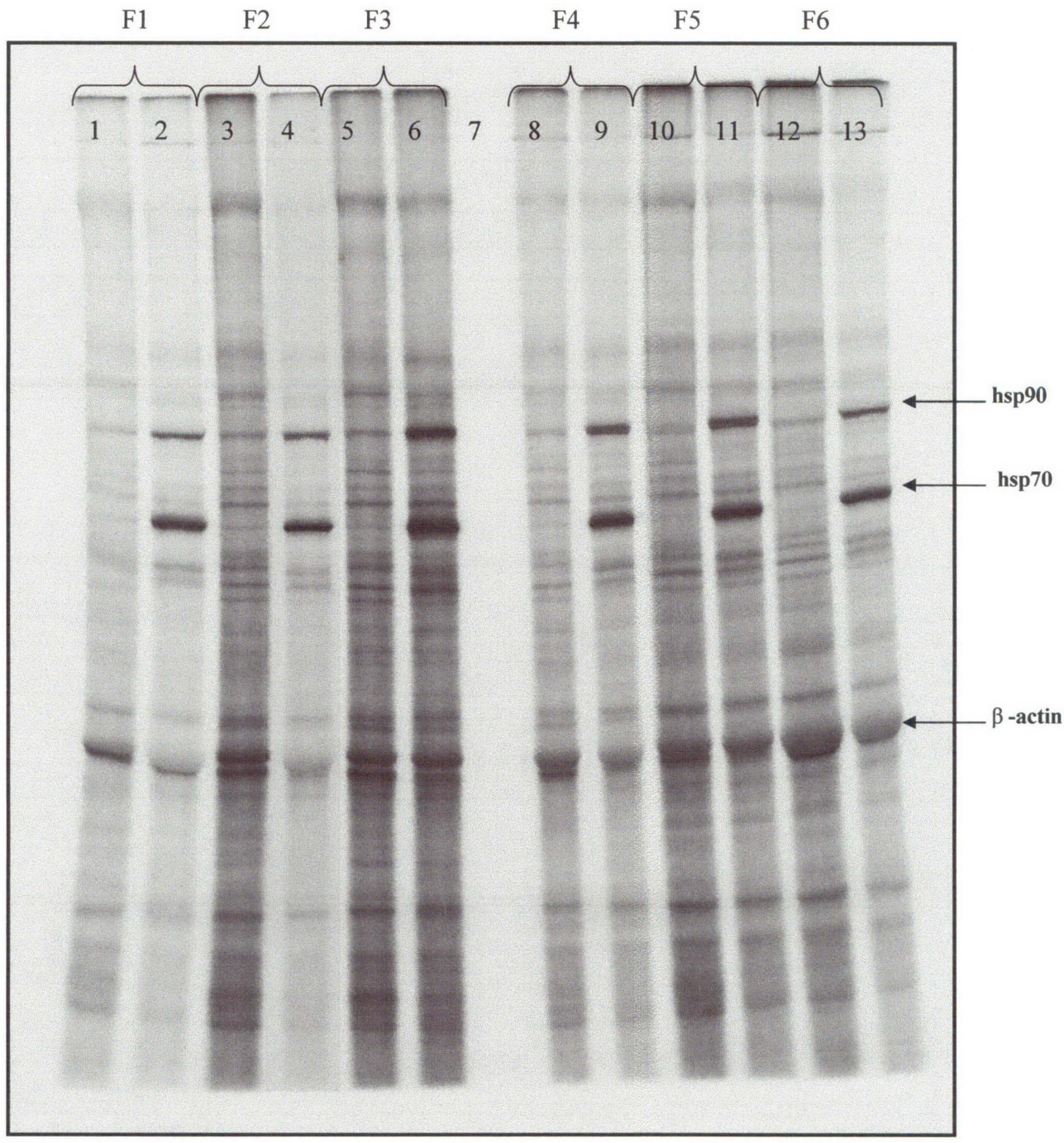


Figure 7.7. Autoradiogram of ^{35}S-methionine labelled lymphocyte proteins from the female cohort post-supplementation with CoQ_{10}.

Lanes 1, 3, 5, 8, 10 and 12: control samples at 37°C throughout for participants F1, F2, F3, F4, F5 and F6 respectively.
Lanes 2, 4, 6, 9, 11 and 13: heat shocked samples at 42.5°C for 1 h followed by a 3 h recovery period at 37°C for participants F1, F2, F3, F4, F5 and F6 respectively.

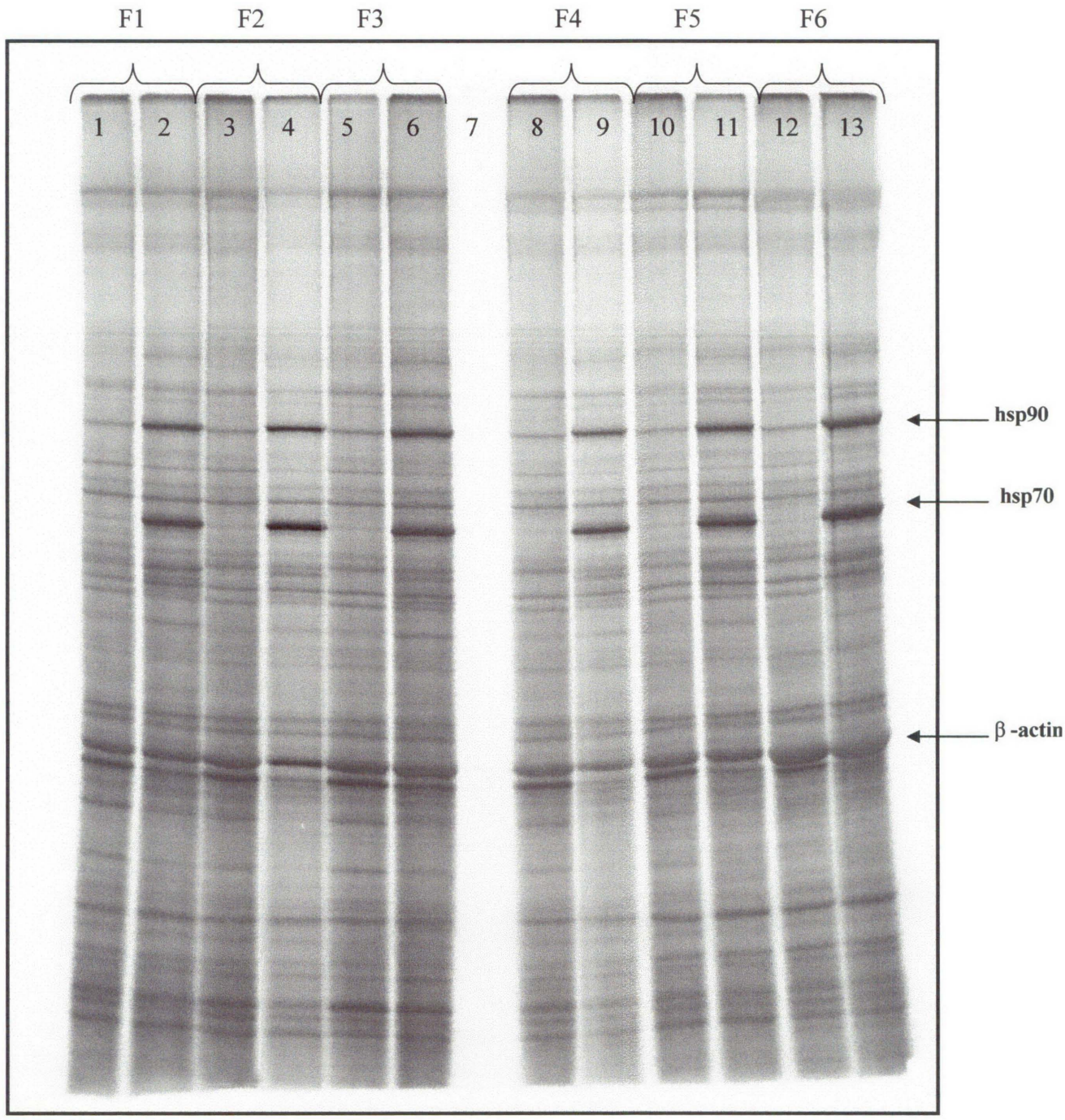


Figure 7.8. Autoradiogram of ^{35}S-methionine labelled lymphocyte proteins from the female cohort post-washout period with CoQ_{10}.

Lanes 1, 3, 5, 8, 10 and 12: control samples at 37°C throughout for participants F1, F2, F3, F4, F5 and F6 respectively.
Lanes 2, 4, 6, 9, 11 and 13: heat shocked samples at 42.5°C for 1 h followed by a 3 h recovery period at 37°C for participants F1, F2, F3, F4, F5 and F6 respectively.

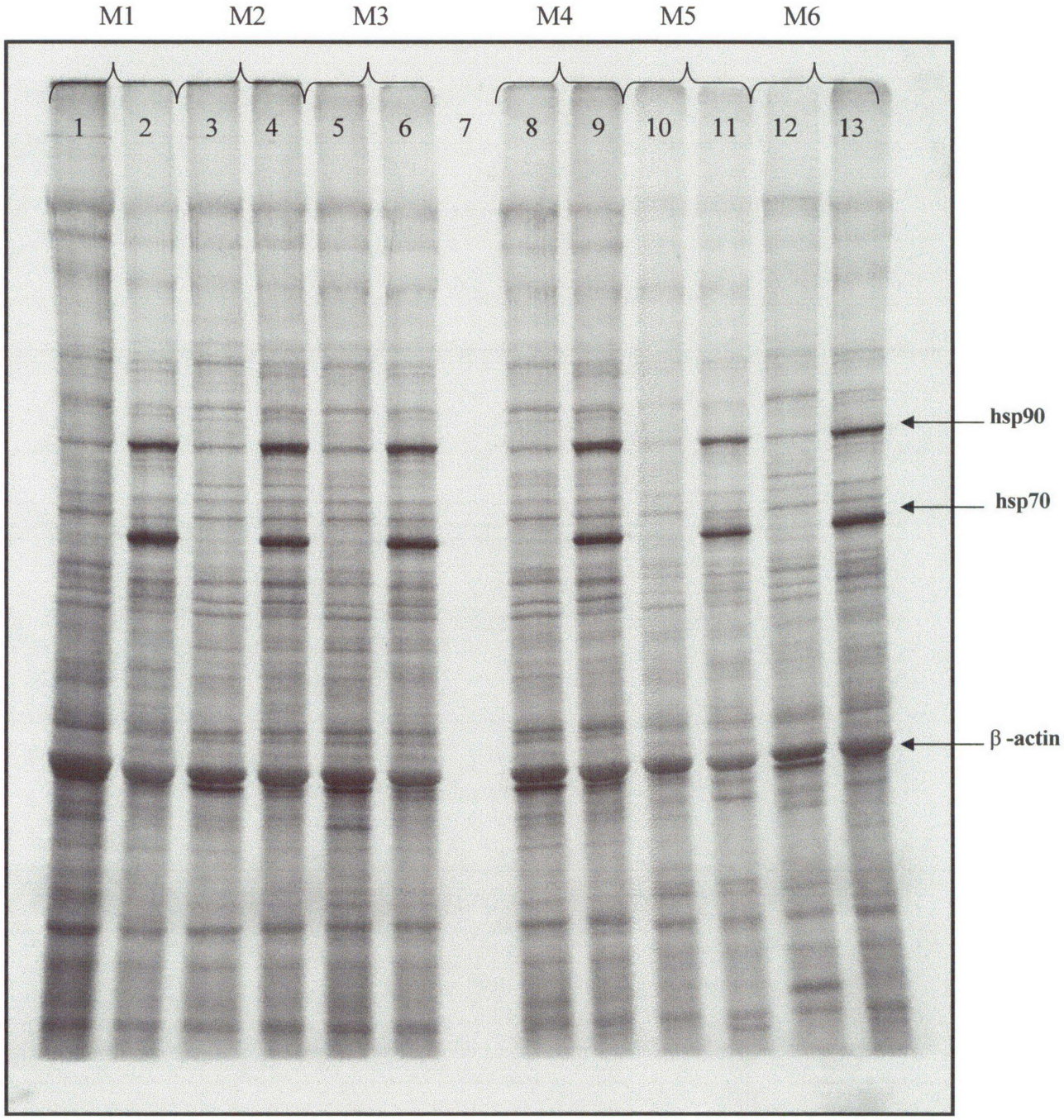


Figure 7.9. Autoradiogram of ^{35}S-methionine labelled lymphocyte proteins from the male cohort pre-supplementation with CoQ_{10}.

Lanes 1, 3, 5, 8, 10 and 12: control samples at 37°C throughout for participants M1, M2, M3, M4, M5 and M6 respectively.
Lanes 2, 4, 6, 9, 11 and 13: heat shocked samples at 42.5°C for 1 h followed by a 3 h recovery period at 37°C for participants M1, M2, M3, M4, M5 and M6 respectively.

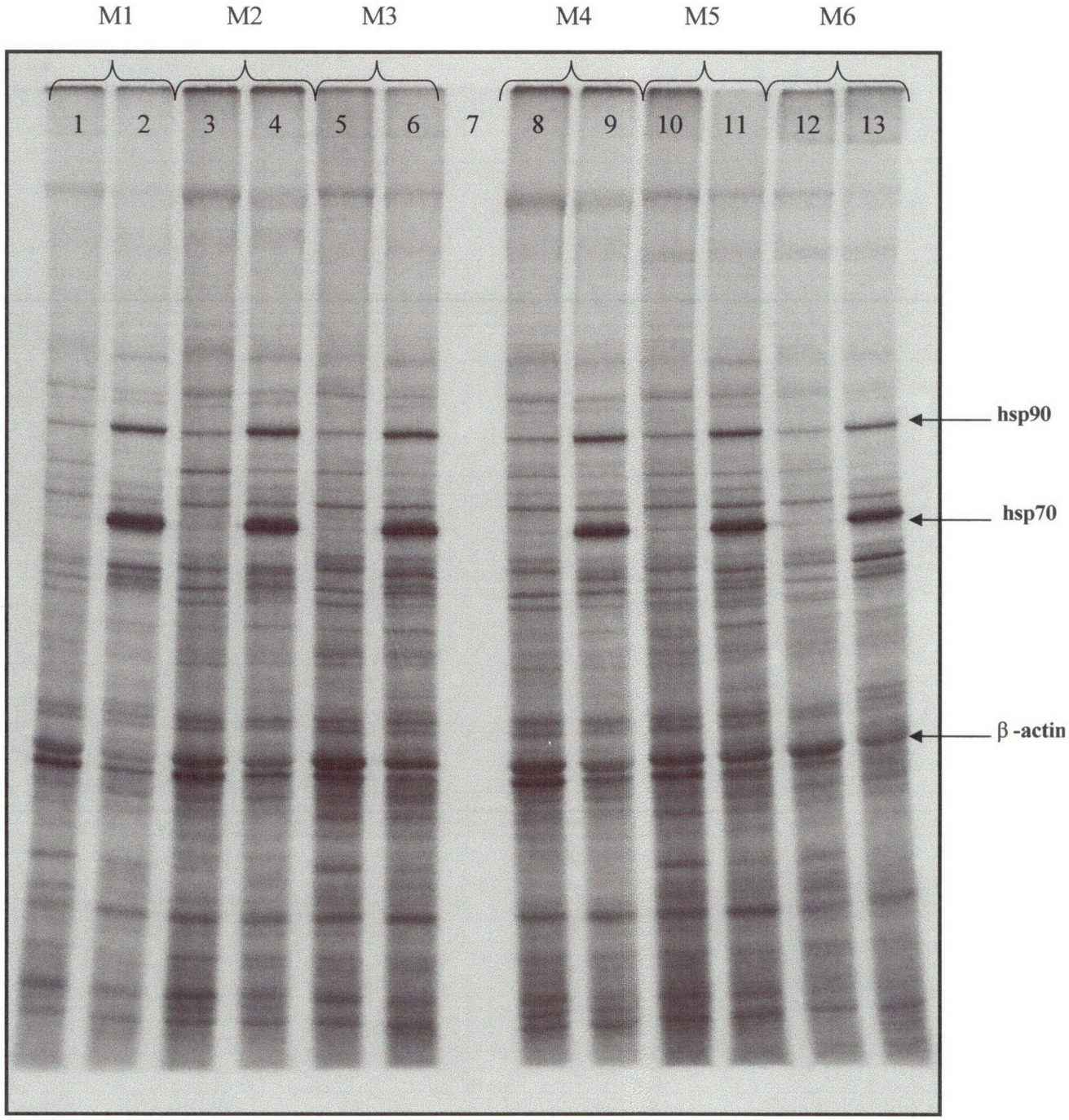


Figure 7.10. Autoradiogram of ^{35}S-methionine labelled lymphocyte proteins from the male cohort post-supplementation with CoQ_{10}.

Lanes 1, 3, 5, 8, 10 and 12: control samples at 37°C throughout for participants M1, M2, M3, M4, M5 and M6 respectively.
Lanes 2, 4, 6, 9, 11 and 13: heat shocked samples at 42.5°C for 1 h followed by a 3 h recovery period at 37°C for participants M1, M2, M3, M4, M5 and M6 respectively.

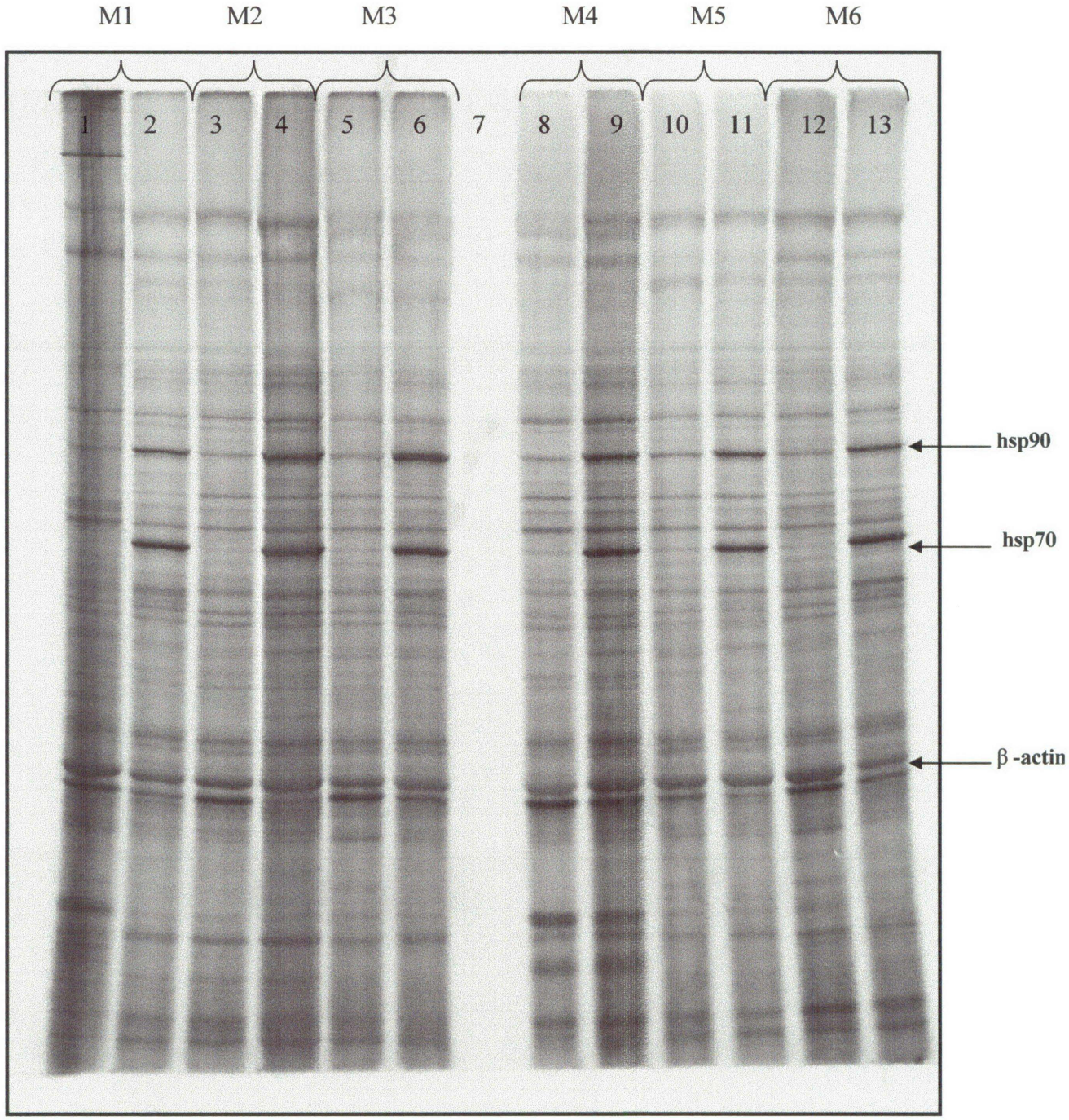


Figure 7.11. Autoradiogram of ^{35}S-methionine labelled lymphocyte proteins from the male cohort post-washout period with CoQ_{10}.

Lanes 1, 3, 5, 8, 10 and 12: control samples at 37°C throughout for participants M1, M2, M3, M4, M5 and M6 respectively.
Lanes 2, 4, 6, 9, 11 and 13: heat shocked samples at 42.5°C for 1 h followed by a 3 h recovery period at 37°C for participants M1, M2, M3, M4, M5 and M6 respectively.

Western immunoblot

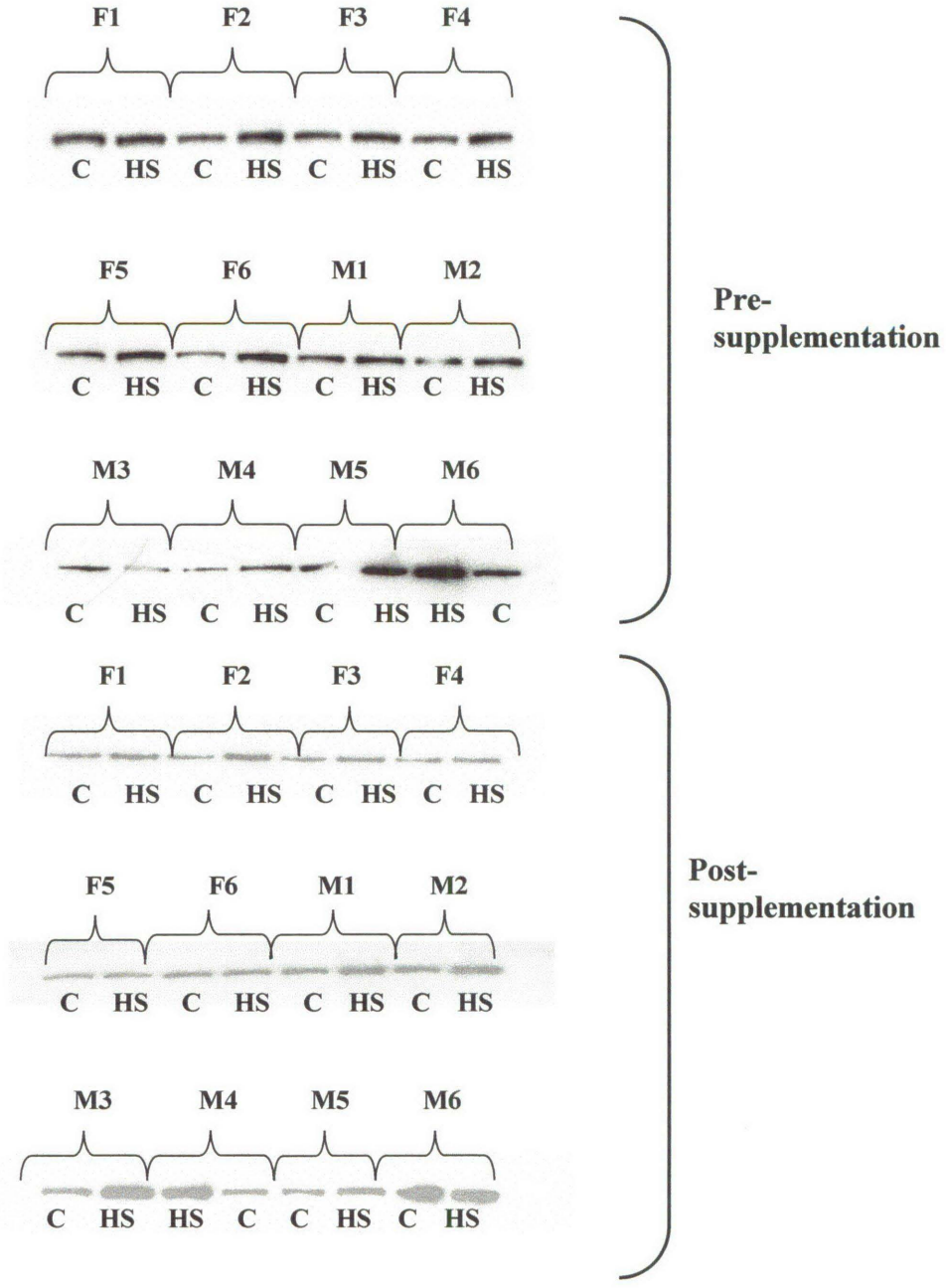


Figure 7.12. Western immunoblots of hsp70 from male and female cohorts, pre-supplementation and post-supplementation with CoQ_{10}.

F1 - F6	: female
M1- M6	: male
C	: control
HS	: heat shock

Table 7.1. Effect of CoQ_{10} supplementation on fold increase of hsp 70 following a heat shock

	Male Cohort	Female Cohort	
	Western Mean ± SD	Western Mean ± SD	*n*
Baseline	1.71 ± 0.50	1.82 ± 0.56	6
Supplemented	1.35 ± 0.30	1.74 ± 0.61	6
Wash out	2.29 ± 0.12	1.81 ± 0.53	6

Male cohort *p*-values for western immunoblot:	Baseline : supplemented	$p = 0.149$
	Baseline : washout	$p = 0.085$
	Supplemented : washout	$p = 0.005$
Female cohort *p*-values for western immunoblot:	Baseline : supplemented	$p = 0.362$
	Baseline : washout	$p = 0.156$
	Supplemented : washout	$p = 0.156$

Table 7.2. Oxidative stress parameters measured in plasma

A: Male

	Baseline Mean ± SD	Supplementation Mean ± SD	Wash-out Mean ± SD	*n*	*p*
Malondialdehyde (µmol/mg protein)	0.96 ± 0.19	0.85 ± 0.90	0.62 ± 0.04	6	0.153 (0.009)
Protein Carbonyl (µmol/mg protein)	14.28 ± 8.77	5.75 ± 1.59	6.16 ± 1.35	6	0.041 (0.083)
Antioxidant Status (mM)	1.28 ± 0.13	1.13 ± 0.40	1.21 ± 0.13	6	0.186 (0.391)

B: Female

	Baseline Mean ± SD	Supplementation Mean ± SD	Wash-out Mean ± SD	*n*	*p*
Malondialdehyde (µmol/mg protein)	0.81 ± 0.16	0.89 ± 0.27	059 ± 0.12	6	0.144 (0.014)
Protein Carbonyl (µmol/mg protein)	19.65 ± 9.32	6.74 ± 1.66	7.28 ± 0.88	6	0.009 (0.017)
Antioxidant Status (mM)	1.16 ± 0.10	1.06 ± 0.12	1.24 ± 0.24	6	0.263 (0.240)

p values are paired student t-test. Values in brackets are baseline versus wash-out, (values not in brackets are baseline and supplemented).

The effect of CoQ_{10} on oxidative stress parameters in plasma

There was no significant difference in the baseline levels of plasma lipid peroxidation, as measured by MDA formation, in the male or female cohorts following CoQ_{10} supplementation [Table 7.2]. There was a significant (p = 0.009 for males; p = 0.014 for females) decrease in MDA formation between baseline levels and levels following a two week wash-out period. Measurement of protein carbonyls, as a measure of protein oxidation, also showed a significant or approaching significant decrease between baseline and wash-out levels (p = 0.083 for males; p = 0.017 for females). In the case of protein carbonyls, there was also a significant (p = 0.041 for males; p = 0.009 for females) decrease following CoQ_{10} supplementation.

Total antioxidant status in plasma was not significantly altered following CoQ_{10} supplementation in either the male or female cohorts. Male values were measured as 1.28 mM pre-supplementation, 1.13 mM after supplementation and 1.21 mM following the wash-out period (p = 0.186). For the females, values were measured as 1.16 mM pre-supplementation, 1.06 mM after supplementation and 1.24 mM following the wash-out period (p = 0.263)[Table 7.2].

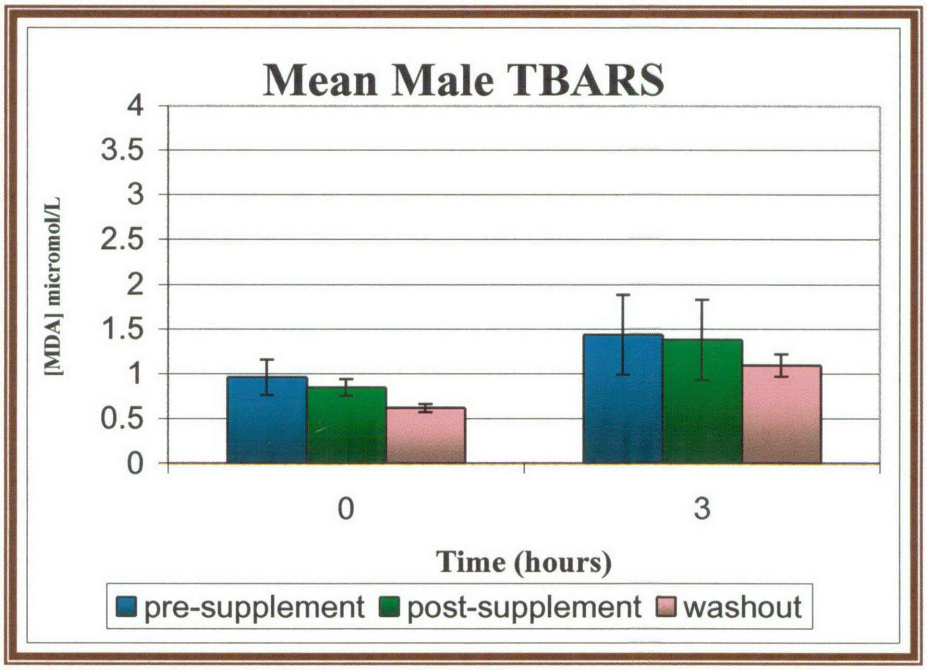


Figure 7.13. Formation of MDA in plasma as an indicator of lipid peroxidation in males, mean ± SD. n = 6. Plasma was exposed to AAPH at 37°C at 0 hr and 3 hr.

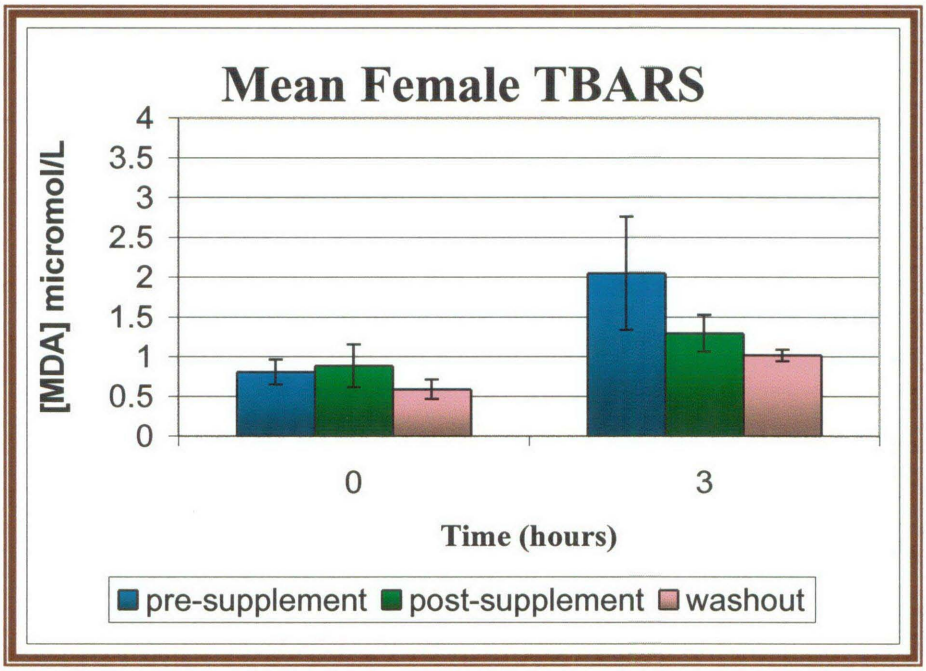


Figure 7.14. Formation of MDA in plasma as an indicator of lipid peroxidation in females, mean ± SD. n = 6. Plasma was exposed to AAPH at 37°C at 0 hr and 3 hr.

Discussion

Coenzyme Q_{10} is one of the most widely used complementary therapies for the treatment of cardiovascular related diseases. Research interest has predominantly focused upon the protective role that the reduced form of CoQ_{10} (ubiquinol) has in pathological and age-related diseases ascribed to either long-term exposure to free radical attack or disturbed mitochondrial function. The vast majority of this research, however, has been on individuals affected by the disease in question with little evidence in regards to healthy individuals. CoQ_{10} has been evaluated for its clinical effectiveness in a number of disease states including various cardiovascular conditions such as atherosclerosis. The role of CoQ_{10} as an antioxidant as well as an essential redox carrier has diversified into research areas to include diseases that are related to ageing such as neurodegenerative diseases. These studies have been prompted by the observation that CoQ_{10} concentration declines with age (Crane, 2001; Turunen *et al.*, 2002). Nevertheless, it is clear that data on healthy individuals is lacking and that such information is required, as CoQ_{10} is advocated as a supplement to promote cardiovascular health.

Dietary intake of a complex combination of nutrients makes it difficult to ascertain relationships between individual nutrients and improved health in either healthy individuals or those suffering from chronic diseases such as cardiovascular disease or cancer. There have been numerous epidemiological studies indicating an inverse relationship associated with the consumption of a diet rich in fruit and vegetables and cancer risk (Ames and Wakimoto, 2002; Fairfield and Fletcher, 2002). This association, however, may be attributed to a variety of anticarcinogenic substances such as fibre and folic acid as well as a variety of antioxidants. In contrast, there have been a number of large-scale studies such as the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ABTS) and the Beta Carotene and Retinol Efficacy Trial (CARET), which have shown that high doses of the antioxidant β-carotene increases the risk of cancer and cardiovascular disease in individuals already at high risk, namely smokers (Bendich, 2004; Goodman *et al.*, 2004). More recently, the Women's Health Study (WHS) did not find any reduction in cancer or cardiovascular events in healthy women taking vitamin E (Lee *et al.*, 2005). Conversely, there have been a multitude of clinical trials indicating a positive association between antioxidants and reduced risk of

diseases such as coronary heart disease (Osganian *et al.*, 2003; Knekt *et al.*, 2004). The positive effects of antioxidants at the cellular level are, however, poorly understood in spite of decades of research.

Ubiquinone in its reduced form functions as an antioxidant preventing lipid peroxidation and is found in such structures as the Golgi apparatus, lysosomes, endoplasmic reticulum, peroxisomes and the plasma membrane. Lipid peroxidation of polyunsaturated fatty acids results in membranes losing their permeability, becoming rigid, reactive and non-functional. Lipid peroxidation produces singlet oxygen, hydroperoxides, lipid epoxides as well as damaging aldehydes such as malondialdehyde (MDA). The latter is a major end product from peroxidation of polyunsaturated fatty acids and assays used to measure MDA formation have become widely used as measure of membrane lipid peroxidation. Plasma concentrations of MDA decreased in both male and females as a result of CoQ_{10} supplementation [Table 7.2]. Although there was no significant difference in plasma MDA levels between baseline and supplementation, there was a significant decrease between baseline and after the two week wash-out period [Table 7.2]. The plausible interpretation for this observation is that CoQ_{10}, a lipid soluble membrane component, requires time to fully integrate into cellular membranes. Thus, any effects of CoQ_{10} on lipid peroxidation would not be apparent until some weeks after supplementation and may have been maintained even after a two week wash-out period.

Protein carbonyl formation is the result of oxidative modification of amino acids in proteins and is commonly used as an early marker of oxidative stress. The observation of decreased protein oxidation subsequent to exogenous oxidative stress challenge in subjects following CoQ_{10} supplementation supports previous studies in both humans and animals indicating an improved cellular defence system following antioxidant supplementation (Barja *et al.*, 1994; Peng *et al.*, 2000; Howard *et al.*, 2002). On the other hand, there was no significant effect on plasma total antioxidant status in either the male or female cohorts following supplementation with CoQ_{10}. However, it should be noted that the assay used in this study is particularly suited to measuring water-soluble antioxidants. This result, therefore, may not be truly indicative of antioxidant status to include membrane-associated antioxidants considering the lipid solubility of CoQ_{10}.

The enzyme NAD(P)H:quinone oxidoreductase catalyses the transformation of CoQ_{10} (oxidised) to $CoQ_{10}H_2$, the reduced form. Genotypic deficiencies in NAD(P)H:quinone oxidoreductase are highly correlated with an increased risk of acute leukaemia in adults (Larson *et al.*, 1999; Smith *et al.*, 2001), childhood acute lymphoblastic leukaemia (Wiemels *et al.*, 1999; Krajinovic *et al.*, 2002a; Krajinovic *et al.*, 2002b) oesophagus and lung adenocarcinoma susceptibility (Lin *et al.*, 1999; Yin *et al.*, 2001), colorectal cancer (Traver *et al.*, 1992; Harth *et al.*, 2000), peritoneal cancer (Fleming *et al.*, 2000), prostatic adenocarcinoma or benign hyperplasia (Steiner *et al.*, 1999), cutaneous basal cell carcinomas (Clairmont *et al.*, 1999), urological malignancies (Schulze-Osthoff *et al.*, 1992) and breast cancer development in women (Marin *et al.*, 1997; Marks-Hull *et al.*, 1997). Very recently it has also been established that NAD(P)H:quinone oxidoreductase stabilises the p53 tumour-suppressor gene (Asher *et al.*, 2001; Asher *et al.*, 2002a) to help explain the enhanced risk of cancer susceptibility in NAD(P)H:quinone oxidoreductase polymorphisms (Asher *et al.*, 2002b). Thus, contemporary evidence indicates that NAD(P)H:quinone oxidoreductase has a protective function in carcinogenesis and cell proliferation.

This study focused upon short-term supplementation with healthy participants supplemented for a two-week period followed by a two-week wash-out. In some of the measured parameters, e.g. alterations in protein carbonyls, post-supplementation levels appeared to have been maintained even after the wash-out period. These results suggest that two weeks may be insufficient for CoQ_{10} levels to return to pre-supplementation levels, possibly due to the lipid solubility of CoQ_{10}.

The effect of CoQ_{10} supplementation on erythrocyte haemolysis

In previous studies, antioxidant supplements were shown to have a protective role on erythrocytes by decreasing erythrocyte haemolysis induced by the azo compound AAPH (Peng *et al.*, 2000; Howard *et al.*, 2002). This test has a number of advantages in monitoring antioxidant defence systems apart from its simplicity and therefore data interpretation. These

are that AAPH breaks down thermally resulting in free radicals biotransformation of enzyme and the rate and generation of this reaction can then be controlled and monitored (Niki, 1990). In the present study, supplementation with CoQ_{10} resulted in an increase in the rate of erythrocyte haemolysis (expressed as $T_{50\%}$) occurring in both the male and female cohorts. Following the washout period, erythrocyte haemolysis produced similar kinetics to post-supplementation rates in the male cohort [Fig 7.4]. A similar profile following supplementation can be seen for the female cohort, however, kinetics of the wash-out period fell back to pre-supplementation rates [Fig 7.5] suggesting the effects of CoQ_{10} supplementation are less sustained in the female cohort. Niklowitz *et al.* (2002) measured CoQ_{10} levels in plasma and erythrocytes in healthy individuals following oral supplementation and noted significant increases in plasma levels of CoQ_{10} after 14 days of supplementation but no significant change in levels in erythrocytes. In another study measuring AAPH induced erythrocyte haemolysis in humans, Cheng *et al.* (2001) reported approximately a 30 min $T_{50\%}$ decrease in individuals following supplementation with a combined antioxidant and vitamin blend. The lipid solubility of CoQ_{10} enables the molecule to integrate into the phospholipid bilayer with the methyl groups of the isoprenoid chain intertwined in the fatty acid chains of the membrane (Genova *et al.*, 2003). The reduced form of CoQ_{10} is more hydrophilic than the oxidised form and thus membrane stability may be lowered in erythrocytes due to changes in the oxidation/reduction of the quinone group.

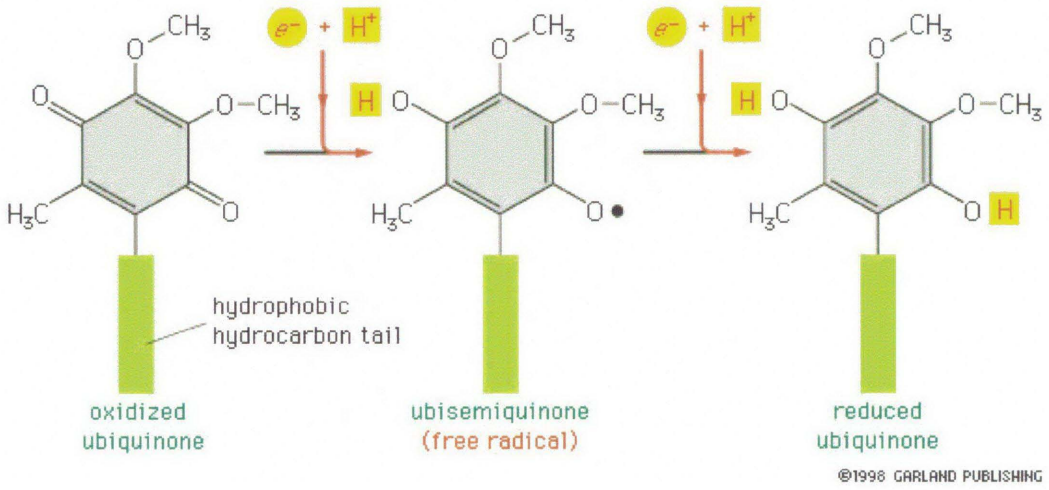


Figure 7.17. Schematic drawing showing the reduction of ubiquinone to ubiquinol via a free radical intermediate.
www.http://fig.cox.Miami.edu/~cmallery/225/225etc/225etc.htm

The chemical reduction of ubiquinone to ubiquinol requires the acceptance and transfer of two single electrons associated with the release or addition of two protons (H^+). Linear electron transfer and transmembranous H^+ translocation occurs within biological membranes. In the mitochondrial membrane, from Complex I or Complex II, ubiquinone is reduced to ubiquinol and subsequently oxidised in two steps, first to ubisemiquinone ($\bullet CoQ^-$) and then to ubiquinol [Fig 7.17]. The ubisemiquinol radical is highly unstable and is thereby capable of transferring an electron to an O_2 molecule resulting in the superoxide ion ($\bullet O_2$) (Nohl *et al.*, 2004). It is also capable of regenerating the vitamin E tocopheroxyl radical by electron donation. Vitamin E is more effective as a mitochondrial antioxidant as it neutralizes lipid peroxyl radicals more readily as well as being less hydrophobic than CoQ allowing greater freedom of movement throughout the mitochondrial membrane (Sohal, 2004). However, unlike CoQ, which is synthesised in the cell, vitamin E must be obtained from the diet. Coupled with vitamin E, CoQ_{10} supplementation has been shown to counteract the prooxidative effect of vitamin E in the active transport of free radicals from aqueous phase into LDL cholesterol due to the preferential location of the redox-active chromanol ring on the outer surface of LDL particles. Thus, not only can the vitamin E tocopheroxyl radical be regenerated by CoQ, but by neutralizing $\bullet CoQ^-$, the likelihood of $\bullet O_2$ generation through autoxidation is lessened (Thomas *et al.*, 1997).

Expression of heat shock protein synthesis in lymphocytes

Heat shock proteins belong to a group of highly conserved and ubiquitous proteins found in both prokaryotic and eukaryotic organisms. Many hsps are expressed constitutively and under non-stressed conditions act as molecular chaperones. Hsps perform essential cellular functions such as protein folding and assembly, suppressing aggregation of non-native proteins and transportation to cellular organelles. It is well known that heat shock and oxidative stress share some common mechanisms, in particular, some heat inducible stress proteins are also inducible by oxidative stress (Morgan *et al.*, 1986; Watson, 1990; Godon *et al.*, 1998). Additionally, it is well documented that a mild heat shock (which induces hsp synthesis) protects cells against oxidative stress. Regulation of hsp expression occurs

primarily at the transcription level and is mediated by the heat shock transcription factors (HSFs). The activation of the HSFs, in particular HSF-1, is highly sensitive to oxidative stress and is regulated by endogenous antioxidant mechanisms (Bijur *et al.*, 1999).

There are few studies that have examined the expression of stress protein synthesis in lymphocytes, and this is surprising considering that lymphocytes are physiologically exposed to high levels of oxidative stress at sites of inflammation. In two small-scale clinical trials, healthy subjects supplemented with different antioxidant complexes have shown significant increases in lymphocyte hsp70 synthesis (Peng *et al.*, 2000; Howard *et al.*, 2002). Furthermore, modulation of hsp70 expression in lymphocytes is indicative of an improved stress response as well as an improved immune response (Agnew *et al.*, 2003). In other studies, it has been reported that hsp synthesis (hsp60, hsp70 and hsp90) in lymphocytes from elderly individuals is attenuated (Rao *et al.*, 1999). It was this and the above observations on oxidative stress that prompted the present studies on hsp expression and CoQ_{10} supplementation as it is also known that CoQ_{10} concentrations decline with age (Crane and Navas, 1997).The question posed was, therefore, whether CoQ_{10} supplementation would alter hsp expression. However, under the present experimental protocols, no obvious alterations in hsp expression, specifically hsp70, was observed in the male or female cohorts.

It is established that mitochondrial function declines with age (Crane and Navas, 1997) and there is some advocacy for CoQ_{10} supplementation in the elderly, albeit with scant clinical evidence for efficacy. Nevertheless, it would be of interest to test CoQ_{10} supplementation in the elderly with respect to hsp synthesis. The present studies showed no significant increases of hsp70 expression in lymphocytes following supplementation in healthy individuals with CoQ_{10}. Although there is some evidence of increases in blood levels of IgG and T4-lymphocytes following CoQ_{10} supplementation (Folkers *et al.*, 1988; Folkers *et al.*, 1991), our results indicate no enhancement of the stress response in lymphocytes in healthy individuals.

Decline in functional mitochondria and CoQ_{10} with age is most damaging to organs that have the highest energy demands, namely, the heart, kidney, brain, liver, and skeletal muscle, in that order (Luft and Landau, 1995). Mitochondrial dysfunction in brains of

individuals with Alzheimer's disease is commonly observed and therapy now including the B-vitamins that act as coenzymes in the respiratory chain (Walker and Byrne, 1995). In addition, clinical trials evaluating the efficacy of CoQ_{10} on Friedreich's ataxia, Huntington's and Parkinson's disease have shown some beneficial effects (Beal, 2004).

In summary, the results of this small-scale study indicated that supplementation with CoQ_{10} improved plasma antioxidant defences as determined by decreased lipid peroxidation and protein oxidation supporting previous observations of the antioxidant properties of CoQ_{10} (Stocker *et al.*, 1991; Forsmark-Andree *et al.*, 1995; Gotz *et al.*, 2000; Niklowitz *et al.*, 2002; Genova *et al.*, 2003; Gómez-Díaz *et al.*, 2003). There was no indication of an improved stress response by altered hsp70 expression in lymphocytes as well as no protective effects on oxidative stress induced erythrocyte haemolysis. On the contrary, the results suggested a prooxidative effect on erythrocyte membranes with respect to free radical induced haemolysis [Figs 7.4 and 7.5]. It has only recently been recognised that CoQ may also act as a pro-oxidant under certain conditions. Nohl *et al.* (2003) have described conditions in liposomal membranes under which the production of ubisemiquinone as an oxidative product of ubiquinone-ubiquinol [Fig 7.17] gives rise to superoxide-derived H_2O_2 leading to membrane lipid peroxidation. The increased erythrocyte haemolysis, indicative of free radical induced lipid peroxidation, following CoQ_{10} supplementation as observed in the present studies may be related to these observations. Interestingly, a number of other antioxidant components have also recently been recognised as having pro-oxidant roles and these include β-carotene, α-tocopherol, vitamin C and α-lipoic acid (Zhang and Omaye, 2001; Çakatay *et al.*, 2005).

CHAPTER 8

Summary & Future Directions

Yeast taxonomy

The genesis of the current study was the isolation and characterisation of a number of yeasts originating from samples of soil, snow, ice and organic material taken from the uniquely diverse Vestfold Hills region of Antarctica. Sixty yeasts were isolated using classical isolation methods. These yeasts were grouped together according to morphological characteristics and whole cell protein profiles. Molecular techniques characterised the yeast isolates with the identification of a novel species - *Cryptococcus watticus* (Chapter 3). Additionally, two Ascomycetous yeast-like isolates had unique D1/D2 sequences differentiating them from all other yeasts in the GenBank database. These isolates proved challenging to characterise with fastidious growth requirements and were, therefore, forwarded on to the relevant expert on Ascomycetous taxonomy at the Centraalbureau voor Schmmelcultures, Utrecht, The Netherlands (Chapter 4).

Along with the isolation of novel yeast strains, a further eight isolates of *Cr. nyarrowii* were recovered. This yeast was isolated for the first time in 2001 from the Vestfold Hills region. The number of new isolates of this species, in combination with the various locations from which it was recovered, indicated that this species may be a truly indigenous Antarctic yeast. Traditionally, the identification of yeasts relied upon observable morphological and physiological features. This has resulted in the misidentification of many yeast isolates. Molecular techniques have provided a powerful taxonomic tool for phylogenetic purposes and comprehensive studies applying these techniques to the classification of Basidiomycetous and Ascomycetous yeast have been published (Kurtzman and Robnett, 1997, 1998; Fell *et al.*, 2000). Yeast systematics has progressed rapidly due to these techniques ensuring relatively prompt and accurate identification. However, although molecular technology provides an

efficacious means of identification, there has been a paucity of data relating to yeast characteristics, interaction between yeasts and environmental influences. Thus, while molecular techniques have revolutionised yeast taxonomy, the classical methods of e.g. cellular morphology, biochemical tests, ubiquinone analysis, fatty acid analysis and cell wall composition, continue to contribute useful data regarding individual species.

Potential commercial applications of Antarctic yeasts

The two classes of essential fatty acids, Ω-6 and Ω-3 are primarily found in fish with a high oil content. This study revealed relatively high percentages of Ω -3 and Ω-6 fatty acids found in *C. parapsilosis* and *Cr. nyarrowii* respectively. There is a large body of evidence indicating the positive effects that Ω -fatty acids have on the health of individuals as well as on a variety of pathological conditions. Arachidonic acid, an Ω-6 fatty acid, is converted into inflammatory prostaglandins and leukotrienes. This conversion is inhibited by Ω-3 fatty acids resulting in significant decreases in leukotrienes, prostaglandins, interleukins and tumour necrosis factor as well as producing an antithrombotic effect due to the decreased production of thromboxane A2 (Oh, 2005). The most compelling evidence of the clinical benefits of Ω-fatty acids comes from research on cardiovascular disease. Epidemiological evidence has been supported by clinical trials that have shown that a diet rich in Ω-3 fatty acids are positively associated with a reduction in cardiovascular mortality, myocardial infarction and sudden cardiac death (Oh, 2005). The National Institute of Health (NIH) and the American Heart Association (AHA) have both issued guidelines advocating a daily intake of between 650-1000 mg/day of Ω-3 enriched foods. However, in 2004 the Food and Drugs Administration (FDA) released a statement advising of the risk of the harmful effects of mercury found in various types of fish and shellfish, recommending pregnant women, women who may become pregnant, nursing mothers and young children to avoid certain types of fish (FDA and EPA, 2004). The relatively high percentages of Ω -3 and Ω-6 fatty acids found in *C. parapsilosis* and *Cr. nyarrowii* respectively, indicate the potential of exploiting these strains for production of these commercially important Ω-fatty acids for use as dietary supplements without the risk of mercury contamination.

Enzymes produced by organisms from extreme environments are highly exploitatable in the biotechnology industry. Cold-adapted enzymes are characterised by increased

efficiency and decreased temperature stablilty compared with their mesophilic and thermophilc counterparts (Georlette *et al.*, 2004). This reduction in stability is thought to be due to a highly flexible structure required for expeditious conformational changes during catalysis at low temperatures. There are numerous commercial applications for cold active enzymes isolated from microorganisms from cold environments, such as additives in washing detergents and in the food industry (proteases from psychrophiles are used in cheese maturation as well as the dairy industry, fermentation processes as well as meat tenderising). Other applications from cold-adapted microorganisms include: antifreeze proteins and solutes as cryoprotectants, polyunsaturated fatty acids as dietary supplements and for environmental bioremediation (digesters, composting, oil or xenobiotic biodegradation) (Cavicchioli *et al.*, 2002; Feller and Gerday, 2003).

Yeasts are generally recognised as a safe organism which may be expoited for human use, however, there is, somewhat surprisingly, very limited literature on cold-active enzymes from yeasts. In one very recent publication, Birgisson *et al.* (2003) have reported the characterisation of cold-active polygalactouranases from yeasts isolated from frozen environmental samples in Iceland. These enzymes are classified as pectinases which are used in the fruit and vegetable processing industries. Screening Antarctic yeasts for this and other cold-active enzymes (e.g. lipases, proteases) would appear to be an exercise with considerable commercial potential.

Yeast biodiversity

Studies on yeast biodiversity in the harsh Antarctic environment are inadequate. Since the 1960's there has been only sporadic research in relation to yeasts isolated from this unique habitat. Historically, this has been attributed to limitations associated with culturing methods as well as the logistic obstacles that need to be overcome when executing any research in Antarctica. Contamination was and continues to be of prime concern, not only in relation to the environment but also with respect to the samples themselves. Precision and accuracy in sampling and labelling is crucial, as is the recording of such environmental conditions as substrate composition, UV-radiation levels, moisture levels and the presence of any flora, fauna or organic substrates. This information underpins yeast biodiversity studies in the Antarctic. Yeasts usually play the role of saprophytes. Recent reports have suggested, however, that in Antarctic soils, some yeasts provide nematodes with ergosterol, a lipid

produced by yeast that is normally supplied by plants in more temperate climates (Kuenning, 2003). The advent of sophisticated molecular techniques has resulted in an influx of yeasts being described as well as the identification of many taxonomic synonyms. This data has expanded current knowledge of yeast biodiversity in the Antarctic, accrediting baseline data and establishing a more comprehensive picture of yeast biodiversity. As soil organisms are one of the most sensitive environmental biomarkers they are, therefore, invaluable in the study of contaminated or disturbed ecosystems. The techniques of molecular biology together with the culturing of isolates will remain important goals with monitoring of microbial activity providing advance evidence of otherwise imperceptible changes.

The number of yeasts isolated from the Vestfold Hills in this study, as well as previous studies from this laboratory, thus far represents the most comprehensive biodiversity data available for Antarctica (Chapter 5). Screening of Antarctic soil, snow and water is of prime importance. The Center for the Advancement of Genomics is currently characterising microbial diversity in marine and terrestrial environments through their global sampling expedition. To date, 1.3 million new genes and at least 1800 new microbial species have been collected from water samples in the Sargasso Sea, Bermuda (Venter *et al.*, 2004). In this context, however, microbial is limited to mean only bacteria. This project provides a template for a similar project to be conducted screening for yeasts and other eukaryotic microbes. The present study is, so to speak, only the tip of the iceberg with respect to the biodiversity of Antarctic yeasts.

Rhodotorula mucilaginosa

Although Antarctic yeasts have been increasingly isolated from various geographic regions, other than the concept that they may provide soil nematodes with ergosterol, the functional role they play in this environment is still unknown. Particular characteristics attribute to their ability to circumvent such extreme conditions. In the case of *R. mucilaginosa*, resistance to the damaging effects of UVR is a distinct advantage. Results from this study have shown *R. mucilaginosa* to be highly resistant to UVA radiation (Chapter 6). Subsequently, during the course of this candidature, opportunity arose through a collaboration with the Australian Institute of Marine Science to examine the coenzyme Q systems of the Antarctic yeasts for taxonomic purposes as well as a sensitive measure of cellular redox potential and oxidative stress under UVA radiation. Preliminary results showed

a reductive response in cellular CoQ balance, increasing the $CoQH_2$/total CoQ ratio on exposure to UVA radiation. To date, this was the only report of such a response in an eukaryotic organism, thus opening the door for future research using this yeast as a model organism to study the regulation of coenzyme Q redox balance in eukaryotes. This is of particular interest given that many degenerative diseases in humans have been linked to a diminished capacity of ubiquine reduction.

The results of this study indicated that while this strain of *R. mucilaginosa* was highly resistant to UVR, the classic heat shock response (i.e. the increased expression of hsp70) was not responsible for this protection. Increased synthesis of the classical hsps was neither induced under heat shock conditions, nor in response to UVA radiation. Nonetheless, heat shock acquired thermotolerance was observed in *R. mucilaginosa*, a characteristic normally indicative of a heat shock response. Given these results, it can be postulated that the unique CoQ redox response of this yeast to UV-photooxidative stress is a cellular adaptation to the high levels of UVR found in Antarctica.

A number of yeast species are known to have significant amounts of carotenoid pigments. The industrial red yeasts, *R. glutinis*, *Sporidiobolus salmonicolor* and *Phaffia rhodozyma* are used commercially as sources of astaxanthin and it has been shown that production of physiologically significant carotenoids in these yeasts is increased when exposed to UV-radiation. There have been numerous epidemiological studies supporting the theory that carotenoids provide moderate protection from UV-induced erythema (Stahl *et al.*, 2000; 2001). This protective role is attributed to the ability to scavenge ROS generated in photooxidative processes (Heinrich *et al.*, 2002). Given the protective properties that carotenoids exhibit, it is not unreasonable to hypothesis that they may serve as a mechanism of survival for yeasts found in extreme habitats such as Antarctica. To further this research, pigmented Antarctic yeast species need to be fully analysed in relation to the type of pigments that are present. Whilst it may seem obvious to screen for MAA's in Antarctic yeasts, it may be that these yeasts contain novel pigments not previously isolated.

Coenzyme Q

The benefits of antioxidants to health in individuals, either by dietary supply or by supplementation, have become a key issue in the healthcare industry. Antioxidants are also

postulated to have a role in slowing the ageing process. According to the well-accepted *free radical theory of ageing,* the accumulation of oxidative damage caused by ROS is largely responsible for the general decline of health we experience with age (Beckman and Ames, 1998). Accrual of cellular ROS is highly damaging to DNA, proteins and lipids initiating cellular apoptosis cascades. During the normal ageing process, metabolic and antioxidant functions decline further leading to the deterioration of tissue and organs as well as late-onset degenerative diseases (Linnane *et al.*, 1998; Knight, 2000; Holbrook and Ikeyama, 2002).

Coenzyme Q_{10} is one of the most popular complementary therapies for the treatment of cardiovascular related diseases. Research interest has predominantly focused upon the protective role that the reduced form of CoQ_{10} has in pathological and age-related diseases ascribed to either long-term exposure to free radical attack or disturbed mitochondrial function. The vast majority of this research however, has been on compromised individuals with little evidence in regards to healthy individuals. CoQ_{10} is the only lipophilic antioxidant to be biosynthesized and has been evaluated for its clinical effectiveness in a number of disease states including various cardiovascular conditions (such as atherosclerosis) and age related diseases (such as neurodegenerative diseases) as it has been well documented that CoQ_{10} concentration declines with age (Crane, 2001; Turunen *et al.*, 2002). The dual role of CoQ_{10} as an antioxidant and as an essential redox carrier has diversified research directions. More data on healthy individuals is warranted as CoQ_{10} is advocated as a supplement to promote cardiovascular health, not only as a treatment for existing disease.

The results of this study indicate that supplementation with CoQ_{10} improved plasma antioxidant defences by deceased lipid peroxidation and protein oxidation supporting observations from other studies of the antioxidant properties of CoQ_{10} (Stocker *et al.*, 1991; Forsmark-Andree *et al.*, 1995; Gotz *et al.*, 2000; Niklowitz *et al.*, 2002; Genova *et al.*, 2003; Gómez-Díaz *et al.*, 2003). There was no indication of an improved stress response by altered hsp70 expression in lymphocytes nor on erythrocyte membranes. On the contrary, our results suggested a prooxidative effect on erythrocyte membranes. These observations raise the important issue that antioxidants may, under certain conditions (e.g. in different membrane-associated environments), act as pro-oxidants generating ROS. Intake of dietary supplements, currently practised by ~60% of the Australian adult population, should, therefore, be carefully monitored.