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Cellular Redox Activity of Coenzyme Q_{10}: Effect of CoQ_{10} Supplementation on Human Skeletal Muscle

ANTHONY W. LINNANE[a,*], GEORGE KOPSIDAS[a], CHUNFANG ZHANG[a], NATALIA YAROVAYA[a], SERGEY KOVALENKO[a], PENNY PAPAKOSTOPOULOS[a], HAYDEN EASTWOOD[a], STEPHEN GRAVES[b] and MARTIN RICHARDSON[c]

[a]*Centre for Molecular Biology and Medicine, Epworth Medical Centre, 185-187 Hoddle Street, Richmond, Vic. 3121, Australia;* [b]*Orthopaedic Department, Royal Melbourne Hospital, Gratten Street, Parkville, Vic. 3052, Australia;* [c]*Epworth Medical Centre, 173 Lennox Street, Richmond, Vic. 3121, Australia*

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In this paper, we report results obtained from a continuing clinical trial on the effect of coenzyme Q_{10} (CoQ_{10}) administration on human *vastus lateralis* (quadriceps) skeletal muscle. Muscle samples, obtained from aged individuals receiving placebo or CoQ_{10} supplementation (300 mg per day for four weeks prior to hip replacement surgery) were analysed for changes in gene and protein expression and in muscle fibre type composition. Microarray analysis (Affymetrix U95A human oligonucleotide array) using a change in gene expression of 1.8-fold or greater as a cutoff point, demonstrated that a total of 115 genes were differentially expressed in six subject comparisons. In the CoQ_{10}-treated subjects, 47 genes were up-regulated and 68 down-regulated in comparison with placebo-treated subjects. Restriction fragment differential display analysis showed that over 600 fragments were differentially expressed using a 2.0-fold or greater change in expression as a cutoff point. Proteome analysis revealed that, of the high abundance muscle proteins detected (2086 ± 115), the expression of 174 proteins was induced by CoQ_{10} while 77 proteins were repressed by CoQ_{10} supplementation. Muscle fibre types were also affected by CoQ_{10} treatment; CoQ_{10}-treated individuals showed a lower proportion of type I (slow twitch) fibres and a higher proportion of type IIb (fast twitch) fibres, compared to age-matched placebo-treated subjects. The data suggests that CoQ_{10} treatment can act to influence the fibre type composition towards the fibre type profile generally found in younger individuals.

Our results led us to the conclusion that coenzyme Q_{10} is a gene regulator and consequently has wide-ranging effects on over-all tissue metabolism. We develop a comprehensive hypothesis that CoQ_{10} plays a major role in the determination of membrane potential of many, if not all, sub-cellular membrane systems and that H_2O_2 arising from the activities of CoQ_{10} acts as a second messenger for the modulation of gene expression and cellular metabolism.

Keywords: Coenzyme Q_{10}; Skeletal muscle; Gene expression; Muscle fibre; Proteomics; Ageing

INTRODUCTION

The literature is replete with reports on the efficacy of CoQ_{10} for the treatment of a wide range of apparently unrelated diseases. These reports are largely anecdotal and embrace a diverse range of pathologies. For instance, CoQ_{10} has been used for the treatment of congestive heart failure,[1] muscular dystrophy,[2] chronic fatigue syndrome,[3] breast cancer[4] and primary biliary cirrhosis.[5] Coenzyme Q_{10} has also been used in a number of amelioration therapies such as support for AIDS patients treated with AZT[6] and improved immune function.[7] With such a diverse range of apparent therapeutic effects, it has been difficult to reconcile the mechanism by which CoQ_{10} acts by the extant description of CoQ_{10} as simply being active in mitochondrial energy metabolism and its action as an antioxidant. In this

*Corresponding author. Tel.: +61-3-94264200. Fax: +61-3-94264201. E-mail: tlinnane@cmbm.com.au.

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communication, we develop a new hypothesis involving CoQ_{10} function. It arises from our study of *vastus lateralis* muscle obtained during the course of a clinical trial conducted on the effect of CoQ_{10} administration on human subjects undergoing surgical hip replacement treatment. In this paper, a comparison of gene and protein expression patterns and of muscle fibre differences between placebo- and CoQ_{10}-treated subjects show that CoQ_{10} regulates the expression of numerous genes.

To explain these findings, we have developed the hypothesis that CoQ_{10} redox poise plays a key role in modulating the redox potential of a wide range of sub-cellular membrane compartments. The different induced sub-cellular membrane potentials in turn, lead to the regulation of localised metabolic fluxes. It follows that the effect of CoQ_{10} administration and its modulation of the redox state in tissues will not be restricted to a limited number of metabolic pathways, and consequently will affect a wide range of tissue activities and disease processes via small intrinsic sub-cellular metabolic perturbations. As a part of this process, we suggest that CoQ_{10} acts to regulate gene expression by way of redox modulation and superoxide formation and conversion to H_2O_2 production, utilising this known mitogen as a second messenger. This hypothesis may be sufficient to explain the process by which CoQ_{10} administration can have a wide range of apparently diverse beneficial therapeutic outcomes.

MATERIAL AND METHODS

Clinical Trial

Consenting informed patients scheduled for hip replacement surgery were enrolled in the trial, four weeks prior to surgery. The patients were randomly divided into two groups and administered placebo or CoQ_{10} soft gel capsules for a period of 24–28 days prior to surgery. Three capsules were administered along with the breakfast and evening meal. The CoQ_{10} (50 mg) was dispersed in soybean oil (279 mg), hydrogenated vegetable oil (82.5 mg), and beeswax (27.5 mg) per capsule; the placebo capsules were identical, except for the omission of CoQ_{10}. Human *vastus lateralis* muscle samples were obtained during surgery from individuals encompassing a range of ages and were asymptomatic for mitochondrial disease. The samples were obtained from the level of the intertrochanteric line of the femur during the course of the standard surgical procedure, immediately dissected to remove all connective tissue, snap-frozen in liquid nitrogen and stored at −80°C until required for analysis. The trial was conducted with the ethics approval of the three hospitals involved.

Protein Preparation for Proteome Analysis

Frozen muscle samples (approx. 300 mg) were ground under liquid nitrogen and homogenised in 40 mM Tris containing a Protease inhibitor Cocktail (Sigma E-8340). Endonuclease (300 units, Sigma E-8263) was added to the homogenate and incubated for 30 min at room temperature. The homogenate was then centrifuged at 100,000*g* for 60 min at 4°C to separate the cytosolic (supernatant) and other proteins (pellet). The pellet was re-suspended in 40 mM Tris and re-centrifuged at 100,000*g* for 60 min at 4°C to further extract cytosolic proteins from the pellet. The supernatants from both washes were combined then added to a 10-fold excess of methanol, stored at −70°C for 12 h and then centrifuged at 5,400*g* for 10 min at 4°C. The resulting precipitated protein pellet (Fraction 1) was dissolved with gentle sonication in 8 M urea, 40 mM Tris, 2 mM TBP (tributyl phosphine), 4% (w/v) CHAPS (3-[(3-cholamidopropyl) dimethylamino]-1-propane sulphonate) and 1% (v/v) ampholytes (Bio-Lyte 3/10). The 100,000*g* pellet (Fraction 2) was re-suspended with gentle sonication in 5 M urea, 2 M thiourea, 40 mM Tris, 2 mM TBP, 2% (w/v) CHAPS, 2% (w/v) SB 3-10 (*n*-decyl-*n*, *n*-dimethyl-3-ammonio-1-propane sulphonate), 1% (v/v) ampholytes (Bio-Lytes 3/10) and 0.001% (w/v) Bromophenol.[8,9]

Two-dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis was a modification of the procedure of O'Farrell.[10] First dimension isoelectric focusing (IEF) separation was performed on a Pharmacia Multiphor Electrophoresis unit according to the manufacturer's instructions with Bio-Rad 17 cm IPG strips, range pH 4–7 (150 μg protein loaded) or pH 7–10 (200 μg protein loaded). Following IEF, focusing strips were equilibrated for 20 min with gentle rocking in 6 M urea, 5 mM TBP, 375 mM Tris–HCl (pH 8.8), 20% (v/v) glycerol, 2.5% (w/v) acrylamide 2% (w/v) SDS (sodium dodecyl sulphate) before loading onto 10–20% (w/v) gradient acrylamide slab gels (slab gel dimensions; 23 cm × 21 cm × 1.5 mm) for molecular weight separation. Electrophoresis was performed on a Bio-Rad Protean II electrophoresis unit at 5°C. Gels were run for 2 h at 3 mA per gel, then approximately 16 h at 18 mA per gel. A discontinuous buffer system composed of an anode buffer containing 0.75 M Tris–HCl (pH 8.3) and 0.005% (w/v) sodium azide, and a cathode buffer containing 192 mM glycine, 25 mM Tris–HCl (pH 8.8) and 0.1% (w/v) SDS was utilised. After molecular weight separation, gels were stained with SYPRO Ruby stain (Bio-Rad) according to the manufacturer's instructions and imaged on a Molecular Imager FX (Bio-Rad). Gel matching and analysis was performed with Bio-Rad Melanie 3 software.

RNA Isolation

Total RNA was extracted from skeletal muscle using the Ultraspec™ RNA Isolation System (Biotecx) following the manufacturer's instructions. Tissue samples (200 mg) were homogenised with Ultraspec™ RNA reagent using the FastPrep™ Instrument FP120 (BIO101, CA, USA). The RNA was extracted with chloroform and precipitated with isopropanol. After being washed with 70% ethanol and dried under vacuum, the RNA was dissolved in RNase-free water.

Microarray Analysis

Microarray DNA analysis was conducted on muscle samples from placebo- and CoQ_{10}-treated subjects using the U95A oligonucleotide array (Affymetrix) containing 12,000 annotated human genes. Double-stranded cDNA was synthesised using the SuperScript™ Choice System for cDNA Synthesis (GIBCO BRL Life Technologies). First-strand cDNA was made utilising total RNA by reverse transcription using oligo-dT containing the T7 promoter sequence at its 5′-end. This was followed by second-strand cDNA synthesis. Such cDNA molecules, containing the T7 promoter sequence, were used to generate biotin-labelled RNA by *in vitro* transcription using T7 RNA polymerase with the BioArray™ High Yield™ RNA Transcript Labeling Kit (Enzo). The labelled RNA thus generated from each sample was sent to Research Genetics (Huntsville, AL, USA) where the RNA was fragmented and used to hybridise the U95A microarray. The hybridised array was then stained with the fluorescent dye phycoerythrin conjugated to streptavidin, which has affinity to biotin. After scanning, the expression levels of the genes were represented by the fluorescent intensities. The difference in gene expression levels between CoQ_{10}-treated and placebo samples was measured by Research Genetics using appropriate computer analysis tools.

Restriction Fragment Differential Display Analysis

To compare the gene expression of different samples, the DisplayPROFILE™ system (Display Systems Biotech, Denmark) for differential display analysis (DDA) was used. After synthesis of cDNA from mRNA, the double-stranded cDNA was digested with *Taq*I restriction enzyme, which is a four-base-cutter leaving a 5′-overhanging end. Following digestion, two different specifically constructed DNA adaptors were ligated to the ends of the cDNA fragments. The first adaptor is the extension–protection adaptor (EP adaptor) which has a 5′-overhanging end and an extension protection group on the 3′-end, preventing 3′ to 5′ synthesis which would normally fill in the overhang. The second adaptor (the standard adaptor) was ligated to the other end. PCR was then carried out on these fragments using two different primers. One primer was a fluorescently labelled 0-extension primer that anneals to the ligated EP adaptor. The second primer specifically anneals to the junction between the standard adaptor and the cDNA insert. Three bases of this second primer extend into the cDNA sequence making this primer specific for certain cDNA sequences. To amplify all three base permutations, 64 such primers are required, each for a separate PCR reaction that amplifies a different set of cDNA fragments containing the equivalent three bases. Consequently, a total of 64 PCR reactions are needed to cover all three base permutations. The abundance of PCR products amplified from the fragments represents the level of gene expression. Each PCR reaction was run on sequencing gels (Australian Genome Research Facility, Melbourne, VIC.) generating a PCR product profile (expression window) for each primer. The fluorescent intensity of the PCR products in each expression window was compared between muscle samples from placebo- and CoQ_{10}-treated subjects using GenoTyper computer software (Perkin Elmer). A 3.0-fold or higher difference in mRNA levels was empirically chosen to represent a significant change in gene expression.

Fibre Typing by Myofibrillar Actomyosin ATPase Histochemistry

Muscle tissue was frozen in isopentane, cooled by liquid nitrogen and stored at −80°C until required. Quadriceps muscle is comprised of a mixture of fibres, in proportions that vary in different regions of the muscle. To minimise variation from one subject to another, only *vastus lateralis* specimens from the intertrochanteric line of the femur was used in the study. Serial 6 μm thick cross-sections were cut on a motorized cryostat (Leica) at −20°C, mounted onto 2% APES (3′-aminopropyltriethoxysilane, Sigma)-coated slides. Myofibrillar actomyosin ATPase histochemical staining was conducted within 30 min after sectioning; ATPase activity at different acid and alkaline pH was used to identify different fibre types.

For ATPase staining at acid pH (4.3 and 4.6), the procedure was as described by Brooke and Kaiser[11] and later modified by Hämäläinen and Pette.[12] Briefly, sections were pre-incubated at room temperature for 7 min in a solution consisting of 54.3 mM sodium acetate and 32.6 mM sodium barbital, adjusted with HCl to pH 4.3 or 4.6. After washing twice for 2 min with a solution containing 18 mM $CaCl_2$ and 100 mM Tris–HCl (pH 7.8), the sections were incubated at room temperature for 45 min in a medium containing 4.5 mM ATP, 19.5 mM $CaCl_2$, 116 mM 2-amino-2-methyl-1-propanol (Sigma

alkaline buffer solution 221) at pH 9.4. After three successive 3 min incubations in 11 mM $CaCl_2$, the sections were incubated twice for 1.5 min each in 2% $CoCl_2$ and then rinsed six times for 30 s in 10 mM sodium barbital and once for 2 min in distilled water. After 45 s incubation in 2% (w/v) $(NH_4)_2S$, sections were washed under running tap water for 5 min, dehydrated in ethanol, cleared in xylene, and embedded in DPX mounting medium (Proscience).

For ATPase staining at alkaline pH (9.4), the procedure described by Bancroft and Stevens[13] was used. Sections were incubated for 40 min at 37°C in a solution containing 1 mM ATP in 0.1 M glycine buffer with 170 mM $CaCl_2$ (pH 9.4), rinsed three times for 1 min in distilled water, and incubated at room temperature for 5 min in 2% $CoCl_2$. Sections were then rinsed under running tap water for 5 min, incubated in 2% $(NH_4)_2S$ for 30 s, rinsed under running tap water for 20 min, dehydrated, cleared and embedded as outlined above.

The same areas of the tissue were imaged at three different pH (4.3, 4.6 and 9.4) on the Nikon Microphot-FX photomicroscope with a Spot CCD camera using a 10× objective lens; 10–15 areas of the muscle tissue were imaged randomly depending on the sample size. Muscle fibres were then typed according to the criteria of Staron and Pette.[14] The fibre types I, IIa, IIab, IIb, Ic, IIc, and IIac can be recognised by these procedures. The percentage of different fibre types was counted using Image-Pro Plus software (Media Cybernetics, MD, USA). In general, 1000–1500 muscle fibres were counted per sample to determine percent fibre type in each subject. To simplify assessment of the tissues, all c-fibres (Ic, IIc and IIac) were grouped together, as the percentage of individual c type fibres was low (less than 7% of the total fibres) in all samples analysed.

RESULTS

Proteome Analysis

We analysed the proteome of *vastus lateralis* muscle samples from two placebo-treated and two CoQ_{10}-treated individuals to identify proteins potentially regulated by CoQ_{10}. The proteome analysis focussed on the detection of (a) proteins induced by CoQ_{10} supplementation (present in samples from CoQ_{10}-treated subjects but absent in placebo-treated subjects) and (b) proteins repressed by CoQ_{10} supplementation (absent in CoQ_{10}-treated subjects but present in placebo-treated subjects). It is appreciated that only high abundance proteins would be detected and that the gels are not descriptive of all *vastus lateralis* proteins. Also, the analysis was made on an "all" or "none" basis and did not encompass moderate changes in protein levels. The proteins from each of the gels were classified into one of three groups: (i) specific proteins which appeared only in either the two placebo-treated individuals or only in the two CoQ_{10}-treated individuals; (ii) common proteins which appeared in all four samples (2 placebo/2 CoQ_{10}); and (iii) the so-called non-matched proteins, which appear in 3 samples or less non-specifically between CoQ_{10} and placebo samples.

Approximately 2,000 proteins were visualised in each of the samples (Table I). The combined (overlaid) results of the four samples analysed are summarized in Table II. Of the high abundance proteins detected, 174 proteins were induced by CoQ_{10} and 77 proteins were repressed by CoQ_{10}. Although the results are of a preliminary nature, the data demonstrate that CoQ_{10} is modulating muscle protein expression. Current work is concerned with the expansion of the number of placebo and CoQ_{10} samples being analysed and the characterisation of specific proteins by MALDI-TOF mass spectroscopy.[15]

Gene Expression Analysis

The gene expression in muscle samples from placebo- and CoQ_{10}-treated individuals was assessed using microarray analysis and DDA. Utility of these systems determined whether oral administration of CoQ_{10} to human subjects can affect the gene expression of human skeletal muscle (as

TABLE I Proteomic analysis to determine the effect of oral CoQ_{10} supplementation on the protein expression of *vastus lateralis* muscle: total high abundance proteins

	Gel at pH 4–7		Gel at pH 7–10		
Protein fraction	Fraction 1 (soluble)	Fraction 2 (sediment)	Fraction 1 (soluble)	Fraction 2 (sediment)	Total
Patient 1/Placebo	627	530	667	188	2012
Patient 2/Placebo	713	527	579	174	1993
Patient 3/CoQ_{10}	883	544	639	181	2247
Patient 4/CoQ_{10}	678	627	627	160	2092

Proteins extracted from muscle samples were divided into a soluble (fraction 1) and a 100,000*g* sediment (fraction 2) then separated on two pH gradient electrophoresis gels (pH 4–7 and 7–10) and stained with SYPRS Ruby. The total number of discrete high abundance proteins (spots) identified for each individual is shown (mean 2,086 with a standard deviation of 115).

TABLE II Proteomic analysis of protein expression of *vastus lateralis* muscle: number of proteins affected by oral CoQ_{10} supplementation

	Gel at pH 4–7		Gel at pH 7–10		
Protein fraction	Fraction 1 (soluble)	Fraction 2 (sediment)	Fraction 1 (soluble)	Fraction 2 (sediment)	Total
			Protein occurrence		
Placebo only*†	20	27	22	8	77
CoQ_{10} only*‡	100	33	30	11	174
Common*¶	302	216	248	76	842
Non-matched*§	1112	836	964	245	3157

* The protein gels from four subjects were compared on a spot by spot basis using Melanie computer software.
† Gel images were overlaid and spots that were found only in placebo-treated subjects and absent from CoQ_{10}-treated subjects.
‡ Gel images were overlaid and spots that were found only in CoQ_{10}-treated subjects and absent from placebo-treated subjects.
¶ Gel images were overlaid and spots that were found in all CoQ_{10}-treated and placebo-treated subjects.
§ Gel images were overlaid and spots that were not consistent in all CoQ_{10}-treated and placebo-treated subjects are shown.
‖ Refer to Table I and text for further details.

exemplified by *vastus lateralis* muscle). DDA used to complement the microarray study as it extends the analysis beyond the limits of the array to a wide range of known and unknown muscle specific genes not contained on the U95A array.

Microarray Gene Expression Analysis

Gene expression levels in skeletal muscle of three CoQ_{10}-treated individuals (aged 70, 75 and 76 years) and two placebo subjects (aged 63 and 75 years) were analysed. Each of the three CoQ_{10}-treated individuals was compared with each of the two placebo subjects, generating a total of six comparisons. In all six comparisons, 115 genes showed a differential expression, with 47 genes up-regulated and 68 down-regulated in the CoQ_{10}-treated subjects.

The precise function of many of the genes regulated by CoQ_{10} supplementation are at present unclear with respect to muscle physiology. However, certain genes were considered to be immediately relevant to muscle biochemistry and function. Among the up-regulated genes were: (a) the glutamate receptor protein GluR5 which is involved in neuronal transmission and synapsis development,[16] (b) Fibroblast growth factor receptor N-SAM, which functions in organ development,[17] (c) Protein kinase C-epsilon involved in cell-cycle control and cell-signalling,[18] and (d) Guanylyl cyclase (which synthesises cGMP), the receptor for the redox sensitive[19] second messenger nitric oxide (NO). Together, guanylyl cyclase and NO regulate a number of complex signalling cascades.[20,21]

Genes that were down-regulated included: (a) the TTF-1 interacting peptide 20 (TIP-20), a transcription termination factor-1 interacting peptide that corresponds to FPI-1[22] and LRF[23] (I. Grummt, personal communication) which are known to be involved in transcription, (b) the TR3 orphan receptor; a steroid hormone receptor,[24] (c) the gene regulator hZFH helicase,[25] and (d) the major group rhinovirus receptor, an adhesion molecule.[26] The complete list of genes regulated by CoQ_{10} treatment is currently being evaluated and will be published elsewhere.

Differential Gene Display Analysis

The effect of CoQ_{10} on gene expression was also studied by restriction fragment differential display, using the displayPROFILE™ system (Display Systems Biotech, Denmark). The abundance of PCR products, which represents the level of gene expression, was compared between CoQ_{10}-treated and placebo samples (three pairs) by running DNA sequencing gels. A total of 58 expression windows for three pairs of age-matched CoQ_{10}-treated and placebo-treated samples were analysed. Fifteen fragments were found to be differentially expressed more than 3.0-fold in all three pairs of samples, with 12 up-regulated and 3 down-regulated by CoQ_{10}. A cutoff point of 2.0-fold resulted in the identification of over 600 fragments that were differentially expressed in all three pairs; this analysis is continuing.

DNA fragments that were differentially expressed require cloning and sequencing for specific identification. However, genes can be tentatively identified by determining the fragment size and the particular expression window in which the fragment was located. This information can be analysed making use of software (displayFIT, Display Systems Biotech) to search the displayFIT database (the displayFIT database contains several thousand gene fragments that have been sequenced and classified according to their size, expression window and species). Consequently, some of the 15 genes (3.0-fold cutoff) that were differentially expressed with CoQ_{10} treatment were tentatively identified. Among the up-regulated genes were the myosin heavy chain genes for type IIa and IIb muscle fibre types, adenylate cyclase 9 which synthesises the important global second messenger cAMP, the DNA polymerase ε subunit which is important in DNA repair and replication,[27] and the heat shock protein HSP70

gene HSPA1L involved in stress responses and protein folding. Down-regulated genes included telomerase (important in replicative senescence and development), RNA I helicase (a role in RNA metabolism and in development of various organs including muscle) and glial fibrillary acidic protein (an astrocyte and Schwann cell-specific intermediate filament protein that is often used as an indicator of nerve damage and scar formation).[28] Selected gene fragments are currently being cloned for sequencing and will be published elsewhere.

Muscle Structure and Fibre Type Changes

An important aspect of skeletal muscle physiology is the recognition that muscle is comprised of a number of distinct fibre types, which meet their main physiological energy requirements utilising different metabolic pathways. Type I fibres derive their energy predominantly from mitochondria, type IIa fibres from a balance between the energy generated by the mitochondrion and glycolysis, and type IIb fibres predominantly obtain their energy from the cell's glycolytic activity. The various fibre types are essentially characterised by differences in the type of myosin heavy chain protein; some muscle fibres express only one myosin heavy chain, while others (type c and type IIab fibres) co-express two or more myosin heavy chains.

The different fibre types in muscle sections can be distinguished by evaluation of the myofibrillar actomyosin adenosine triphosphatase (mATPase) activity. The procedure is based upon the observation that fast (type II) and slow (type I) myosins have different alkaline and acid stability. Histochemically, fast muscle fibres display high mATPase activity under alkaline conditions and low activity under acid conditions (alkaline-stable, acid-labile muscle fibres), whereas slow muscle fibres exhibit the reverse (alkaline-labile, acid-stable muscle fibres). This classical histochemical technique allows the recognition of the following fibre types: I, IIa, IIab, IIb, Ic, IIc, and IIac.[14,28]

The muscle fibre type of 14 males that were 57 years and older was evaluated; seven receiving placebo (mean age 65.1 ± 3.3) and seven receiving CoQ_{10} supplementation (mean age 68.3 ± 2.8). Although large variations in fibre type composition were found for both the placebo and CoQ_{10} subjects, a clear pattern was evident. The data indicated that the CoQ_{10}-treated patients have a dramatically different fibre type composition compared to the placebo counterparts (Figs. 1 and 2). The percentage of type I fibres in CoQ_{10}-treated patients was lower than that found in the placebo-treated individuals. Conversely, the proportion of type IIb fibres showed a marked increase in number compared to the placebo-treated individuals, as apparently did the type IIab fibres. The percentage of type IIa fibres in the CoQ_{10} samples did not appear to be different to the placebo. Overall, the placebo samples generally had a much higher proportion of type I fibres and lower proportion of type II fibres, compared to the CoQ_{10} samples. In Fig. 3, the data are presented as the mean percentage of the fibre types. Note, the fibre type composition in human quadriceps muscle alters with age; the percentage of type I fibres increases and the percentage of type II fibres decreases.[29] The CoQ_{10}-treated subjects have a fibre-type composition that is more reflective of muscle from younger subjects. The trial is still in progress; with increased patient numbers, the changes will be quantified.

DISCUSSION

Since the discovery of CoQ_{10},[30] few other compounds have been so intensely studied. A wide range

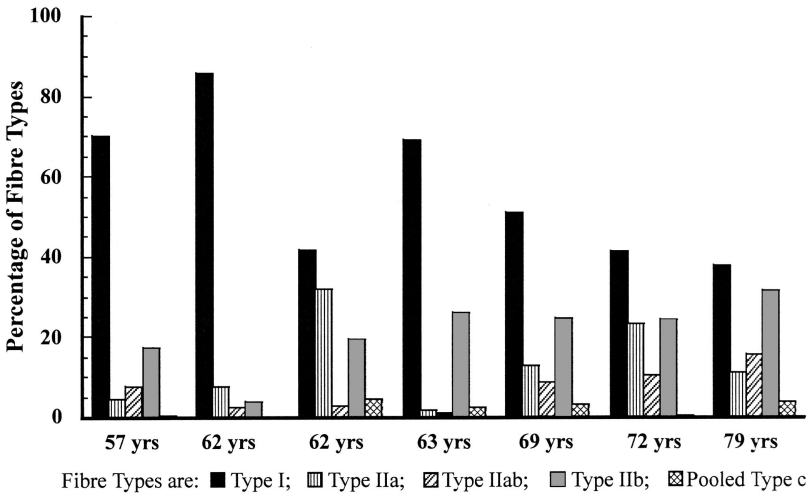

FIGURE 1 Muscle fibre type profiles of placebo-treated patients. Indicated is the proportion of each of the common fibre types found in quadriceps muscle samples taken from individuals of various ages treated with placebo.

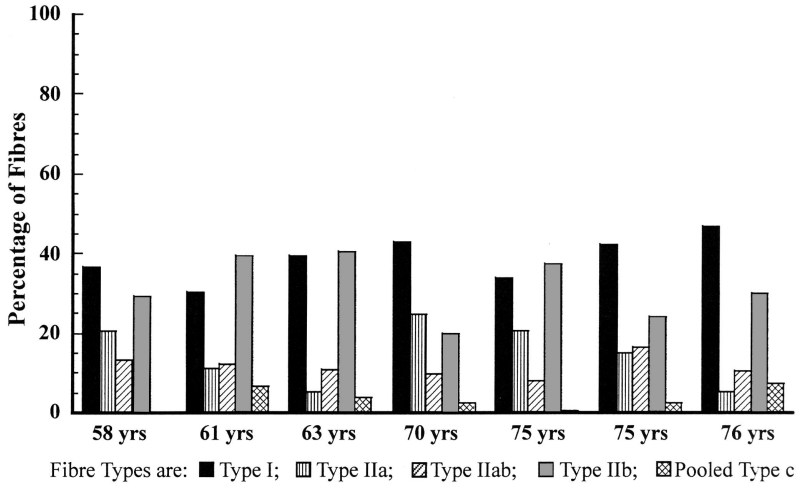

FIGURE 2 Muscle fibre type profiles of CoQ_{10}-treated patients. Indicated is the proportion of each of the common fibre types found in quadriceps muscle samples from seven individuals of various ages treated with CoQ_{10}.

of clinical studies on the effects of CoQ_{10} supplementation have been conducted on a diverse range of pathologies, with apparently beneficial results. The results presented here indicate that CoQ_{10} functions as a major skeletal muscle gene regulator and as such, profoundly modulates cellular metabolism. The expression of a large number of genes is affected by CoQ_{10} as demonstrated independently by micro-array gene expression analyses and differential gene display analyses. Proteome analysis reflects the global gene response of CoQ_{10} supplementation on the protein expression profile of the muscle tissue. Additionally, *vastus lateralis* muscle fibre types were demonstrated to change as a result of CoQ_{10} administration to human subjects; there was a decrease in type I fibres and an increase in type II fibres. The changes were all the more striking as the overall fibre composition following CoQ_{10} administration to older subjects became more reminiscent of the fibre type composition of the muscle of younger subjects. A significant effect of CoQ_{10} administration on skeletal muscle has been earlier demonstrated by our group as being effective in the re-energisation of skeletal muscle of aged rats.[31]

The question arises therefore, as to how CoQ_{10} could possess such wide-ranging effects and act as a gene regulator when it is membrane localised? We suggest that a broad-based cellular redox function by CoQ_{10} may be sufficient to encompass its wide-ranging effects. We propose that CoQ_{10} plays a key role in manipulating the redox potential poise, thereby affecting sub-cellular membrane potential

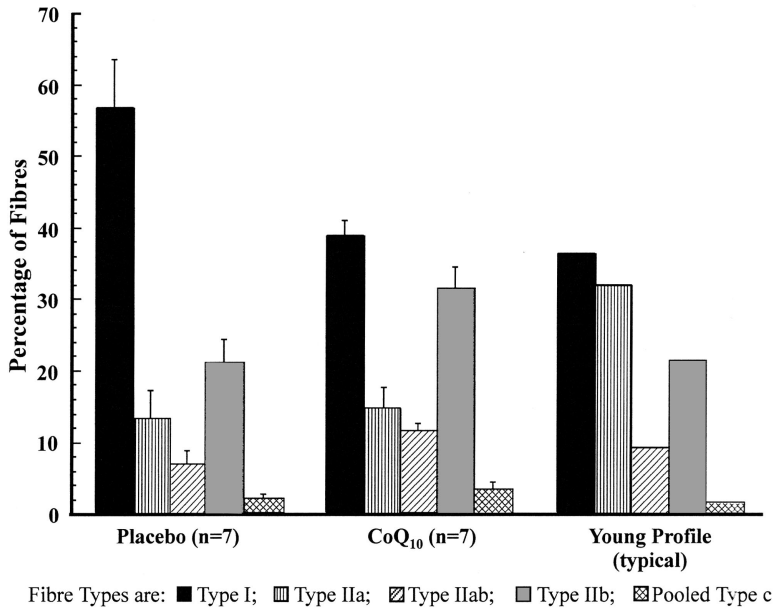

FIGURE 3 Average muscle fibre type profiles of aged placebo-treated and aged CoQ_{10}-treated individuals compared to a typical muscle fibre type profile of a young (<50 yrs) male. Indicated is the average proportion of each of the common fibre types found in quadriceps muscle samples from seven placebo-treated and seven CoQ_{10}-treated subjects.

changes, resulting in the differential regulation of sub-cellular membrane activities and compartments. Different sub-cellular redox poises and their modulation would lead to not only significant localised, but also wide-ranging metabolic changes. Further, superoxide anion (giving rise to H_2O_2), generated by reactions involving CoQ_{10} would reflect specific redox poise, and would play a major role in cellular regulation; H_2O_2 would act as a second messenger in the regulation of gene and proteome expression.

These concepts are derived from the consideration of a range of disparate data, only some of which are outlined here. For instance:(1) chloroplast DNA transcription and the specific products of transcription, have been shown to be regulated by the relative oxidation/reduction level of plastoquinone.[32] It may be hypothesised that mitochondrial transcription is similarly regulated. (2) CoQ_{10} through the Q cycle participates in the determination of mitochondrial membrane potential and in turn energy synthesis and mitochondrial substrate utilisation. Conversely, CoQ_{10} also has recently been shown to be an essential co-factor of the uncoupling proteins which act to down-regulate mitochondrial membrane potential.[33] (3) Nohl and colleagues[34] have recently demonstrated the occurrence of a lysosomal CoQ_{10} oxido-reductase system, which establishes a proton gradient across the membrane. Such a system would contribute to the regulation of metabolite movement in and out of the lysosome. Crane and colleagues[35] have extensively reported on the properties of a CoQ_{10} NADH oxido-reductase enzyme complex in the plasma membrane, which again will contribute to redox potential poise and substrate movement. Further, Crane *et al*.[36] have made a preliminary report on a CoQ_{10} oxido-reductase localised in the golgi membrane complex. We propose that further studies may show that the CoQ_{10} located in other membrane systems reflects undiscovered oxido-reductase systems which will contribute to the determination of individual membrane potentials. (4) CoQ_{10} functions as a H^+/e^- donor through which sulphydryl/disulphide intra-protein crosslinks are converted and in part, determines protein conformations.[37] (5) a key aspect of this proposal is that changes in redox poise would also influence the anti-oxidant/pro-oxidant role of CoQ_{10}. Coenzyme Q_{10} acting as a pro-oxidant, continually gives rise to superoxide anion which superoxide dismutase converts to H_2O_2. Hydrogen peroxide has been shown to function as a mitogen and is involved in the regulation of gene expression.[38] The modulation of redox by CoQ_{10} may act using H_2O_2 as a second messenger.

While H_2O_2 is clearly a strong candidate for a redox-sensitive second messenger, other transient redox-sensitive signalling molecules such as NO may also be important, particularly as NO has also been implicated in contractile function and energy production in cardiac muscle[39,40] and has been shown to activate soluble guanylyl cyclase (shown here to be up-regulated by CoQ_{10}) which synthesises the second messenger, cGMP.

There are an increasing number of processes which are being recognised as subject to redox regulation,[41–43] thus our hypothesis is that CoQ_{10} plays a major seminal role in this overall expression. We propose that CoQ_{10} functions by way of sub-cellular membrane compartmentalised redox poise modulation with the variable generation of the superoxide anion and H_2O_2. In turn, H_2O_2 acts as a second messenger to affect the regulation of gene expression and control of cellular development and metabolism. It follows from our proposition that the effect of CoQ_{10} on cells and tissues will be wide-ranging with the capacity to modulate diverse tissue activities and disease processes via small intrinsic cell metabolic perturbations. It is envisaged that small metabolic imbalances, which may have no short-term untoward effect, may lead to systemic disease when maintained over long periods of time. The development of systemic diseases is inherently slow and develops over decades; systemic disease is uncommon under the age of 50 years. An appropriate compensatory modulation of the processes by CoQ_{10} administration would be predicted to have favourable therapeutic outcomes over a wide range of diseases consistent with numerous reports on the efficacy of CoQ_{10} in a diverse range of pathologies.

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