Coenzyme Q\textsubscript{10} treatment improves the tolerance of the senescent myocardium to pacing stress in the rat

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Abstract

Objective: In elderly patients the results of cardiac interventions are inferior to those in the young. A possible contributing factor is an age-related reduction in cellular energy transduction during the intervention which may induce aerobic or ischemic stress. To investigate whether coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}) improves the response to aerobic stress, functional recoveries of senescent and young rat hearts after rapid pacing were compared with or without CoQ\textsubscript{10}.

Methods: Young (4.8±0.1 months) and senescent (35.3±0.2 months) rats were given daily intraperitoneal injections of CoQ\textsubscript{10} (4 mg/kg) or vehicle for 6 weeks. Their isolated hearts were rapidly paced at 510 beats per minute for 120 min to induce aerobic stress without ischemia.

Results: In senescent hearts pre-pacing cardiac work was 74\% and oxygen consumption (MVO\textsubscript{2}) 66\% of that in young hearts. CoQ\textsubscript{10} treatment abolished these differences. After pacing, the untreated senescent hearts, compared to young, showed reduced recovery of pre-pacing work, (16.8±4.3 vs. 44.5±7.4\%; \textit{P}<0.01). CoQ\textsubscript{10} treatment in senescent hearts improved recovery of work, (48.1±4.1 vs. 16.8±4.3\%; \textit{P}<0.0001) and MVO\textsubscript{2} (82.1±2.8 vs. 61.3±4.0\%; \textit{P}<0.01) in treated versus untreated hearts respectively. Post-pacing levels of these parameters in CoQ\textsubscript{10} treated senescent hearts were as high as in young hearts.

Conclusions: (1) Senescent rat hearts have reduced baseline function and reduced tolerance to aerobic stress compared to young hearts. (2) Pre-treatment with CoQ\textsubscript{10} improves baseline function of the senescent myocardium and its tolerance to aerobic stress.

Keywords: Aging; Cardiovascular surgery; Contractile function; Energy metabolism; Mitochondria

1. Introduction

Over the last 20 years there has been a steady rise in the mean age of patients with ischemic heart disease presenting for interventional therapy [1]. The early mortality for elderly patients after myocardial infarction [2], post-infarction reperfusion [3], angioplasty [4,5] and cardiac surgery [6,7] is up to three times greater than for younger patients, particularly in the urgent setting. In recent years, age has risen in significance as a predictor of mortality for coronary bypass surgery [8], and remains one of the most significant predictors of failure after medical reperfusion [3]. In elderly patients undergoing coronary bypass surgery the commonest cause of death is myocardial failure [9]. However there is little published work on ways of specifically countering the negative effect of age on the ability of the heart to recover from stressful cardiac interventions.

Studies in sheep [10], and rabbits [11] have suggested that the senescent myocardium has reduced tolerance to ischemic stress. The mechanisms underlying this age-related intolerance to stress are not well understood, but impaired mitochondrial function [12] or altered calcium homeostasis with a consequent reduction in cellular energy production have been implicated [13]. Coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}) has emerged as a serious candidate for therapeutic...
use in the amelioration of bioenergetic defects manifested during senescence [16,17]. CoQ\textsubscript{10} is an integral component of the mitochondrial oxidative phosphorylation system, being a lipid-soluble redox carrier between particular respiratory enzyme complexes in the electron transport chain in the mitochondrial inner membrane. Therefore, under conditions where a reduction in mitochondrial respiratory chain function underlies the bioenergetic deficiencies of cells [14,18], it would be anticipated that administration of CoQ would ameliorate the functional shortfall. The logical basis is the restoration of optimal rates of oxidation–reduction reactions in the respiratory chain by enhancing the intramitochondrial concentration of CoQ\textsubscript{10}. Indeed, CoQ\textsubscript{10} is used successfully as a therapy for some patients with neuromuscular and other diseases associated with mitochondrial DNA mutations [16,19]. Much attention is being paid to the clinical use of CoQ\textsubscript{10} in cardiovascular medicine, such as congestive heart failure [17]. At the laboratory level, studies in rats treated with zidovudine (AZT) to induce experimental mitochondrial DNA depletion by limiting oxygen delivery to the heart showed that CoQ\textsubscript{10} supplementation was able to reverse the cardiovascular complications produced by AZT treatment [20].

A better definition of the potential of CoQ for ameliorating age-associated myocardial deficits would be obtained if, in an animal model, it could be demonstrated that administration of CoQ does restore depleted cardiac function associated with senescence. In this study we have used a working heart preparation from laboratory rats [21] to test the following two central propositions: (1) that under appropriate conditions of stressful work the hearts of old rats are functionally debilitated compared to those of young rats; (2) that administration of CoQ\textsubscript{10} to rats will ameliorate age-associated functional deficits in the heart. A corollary of the second proposition is that administration of CoQ\textsubscript{10} should show greater benefit to hearts of old rats than those of young rats. Using working heart preparations from various cohorts of laboratory rats, we first report that the hearts from senescent rats do show a markedly inefficient recovery of pump function after a severe, aerobic, pacing stress, compared to hearts from young rats. Second, we report that pre-treatment of senescent rats with CoQ\textsubscript{10} greatly enhances the performance of their hearts after they had been subjected to aerobic pacing stress.

2. Methods

2.1. Pre-treatment

Young (4.8±0.1 months) and senescent (35.3±0.2 months) female Sprague Dawley rats were randomly assigned to receive daily intraperitoneal injections for 6 weeks (mean 47±1 days) of either CoQ\textsubscript{10} (4 mg/kg) dispersed in a 2% aqueous suspension of polyoxyethylated hydrogenated castor oil (HCO-60), or an equivalent volume (70–100 μl) of HCO-60. Forty-four animals completed the course of treatment and 35 hearts were successfully isolated and perfused. Arterial blood pressure was measured in the awake state using a tail cuff in subgroups of senescent and young rats (n=7 per group).

The dose regime for CoQ\textsubscript{10} (4 mg/kg) was based on reports of clinical usage in chronic trials in man [16], and on a study demonstrating improved exercise tolerance in rats [22]. No side effects were observed. In a subgroup of senescent rats we used CoQ\textsubscript{10} at one tenth of this dose (0.4 mg/kg), and observed no cardioprotective effect (unpublished observations).

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.2. Perfusion

Anaesthesia was induced with 3.5% halothane and oxygen, heparin (1000 units/kg) was administered intravenously and the heart and lungs excised en bloc and placed into ice cold buffer solution containing (in mmol/l): NaCl 118.0, KCl 4.7, CaCl\textsubscript{2} 2.5, MgSO\textsubscript{4}7H\textsubscript{2}O 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 25, (±)-glucose 11.0. The heart was then mounted on an isolated working rat heart apparatus [21] and retrograde perfusion via the aorta was commenced at a pressure of 95 mmHg with buffer equilibrated with 95% O\textsubscript{2}/5% CO\textsubscript{2}. The left atrial appendage was incised and cannulated for the working mode. Pressure measurements were recorded (Grass Polygraph, Model 7, Quincy, MA, USA).

2.3. Pacing stress

After 15 min retrograde (nonworking) perfusion the hearts were made to work for 15 min with a left atrial pressure of 11 mmHg and a mean aortic pressure of 74 mmHg. Myocardial work and oxygen consumption (MVO\textsubscript{2}) were measured.

Work, MVO\textsubscript{2} and efficiency were calculated as follows [23]:

$$W_p = P_{dev} \times CO \times 0.0022$$

where $W_p$ is the power work (mJ/s), $P_{dev}$ is developed pressure (mmHg, systolic aortic pressure minus left atrial pressure) and $CO$ is cardiac output in ml/min (aortic flow plus coronary flow). An appropriate conversion factor for
SI units is included [24]. $\text{MVO}_2$ in $\mu\text{l/g/min}$ was calculated according to the following formula:

$$\text{MVO}_2 = \frac{(P_{O_2} - P_{O_2}) \times 0.024 \times CF \times 1000}{760 \times \text{HW}}$$

where $P_{O_2}$ and $P_{O_2}$ are arterial and venous partial pressures respectively of oxygen (mmHg), 760 is the barometric pressure (mmHg), 0.024 is the solubility coefficient of oxygen in the perfusate at 37°C [25], $CF$ is the coronary flow (ml/min), and $\text{HW}$ is the wet heart weight (g). Mechanical efficiency allows for differences in $\text{MVO}_2$ secondary to altered work capacity. This is calculated by dividing the work by the $\text{MVO}_2$ and expressing the result as a percentage of the expected energy equivalent of complete oxygen combustion, which is $20.97$ Joules/ml of $O_2$ [24].

Myocardial efficiency = $\frac{W_p \times 60 \times 100}{\text{MVO}_2 \times 20.97}$

Work is expressed as mJ/s/g, and $\text{MVO}_2$ as $\mu\text{l/min/g}$. Recovery of work, $\text{MVO}_2$ and efficiency after pacing were expressed as percentages of pre-stress values.

Following baseline assessment of work and $\text{MVO}_2$, the heart was returned to retrograde perfusion and subjected to a stress of 120 min of ventricular pacing (Grass SD9 Stimulator), at 510 beats per minute (bpm, unpaced rate young, 249±9, senescent, 205±5 bpm), during Langendorff mode perfusion. Coronary flow and $\text{MVO}_2$ were measured at 15 min intervals throughout the pacing period. Following pacing, the heart was perfused for a final 15 min in the working mode. At the end of the perfusion, hearts were freeze-clamped, and stored in liquid nitrogen at $-190°C$, for later assay of high energy phosphates.

2.4. Coronary and myocardial lactate

In an additional 25 rat hearts (12 young and 13 senescent), lactate release was measured to determine whether pacing stress in this preparation induced ischemia. It was found that a twofold increase in heart rate to 510 bpm (resting rate approximately 230 bpm) was the maximum rate compatible with a stable preparation. Lactate release into the coronary effluent was measured at four time points: during the pre-stress working period, 5 and 90 min after the onset of pacing and finally in the post-stress working period. Coronary effluent was collected and stored in pre-cooled Eppendorf tubes at $-70°C$, then subjected to enzymatic assay of lactate [26]. The hearts were freeze-clamped at the end of perfusion and extracted with perchloric acid for assay of myocardial lactate.

2.5. Creatine phosphate and ATP assay

Frozen ventricular tissue was homogenised with 1 M perchloric acid and centrifuged. The supernatant was neutralized with 5 M KOH and centrifuged. Hexokinase was added producing NADPH from NADP, and measured by spectrophotometer (CobasBio, Roche) at 340 nm. Finally creatine kinase was added to assay creatine phosphate [27].

2.6. Statistical analysis

Results are expressed as the mean±standard error of the mean (S.E.M.). Two-way analysis of variance (ANOVA) was used to compare differences between the groups and determine interaction effects. Differences between group pairs were compared by the unpaired Student’s t-test. ANOVA for repeated measures was used to test for differences between treatment and age over time for $\text{MVO}_2$ and coronary flow. $P<0.05$ was considered statistically significant.

3. Results

3.1. Body and heart weight

Senescent rats ($n=19$) had a significantly greater body weight than young rats ($n=16$) (359.0±11 vs. 287±6 g: $P<0.0001$). Senescent rats also had a significantly greater heart weight than the young (1.35±0.07 vs. 0.92±0.03 g: $P<0.0001$). The heart to body weight ratio was not significantly different between the senescent and young rats (3.6±0.2 vs. 3.1±0.1 mg/g respectively; $P=0.055$). There were no significant effects of CoQ$_{10}$ treatment on heart weight, body weight or (heart weight)/(body weight). The systolic blood pressure measured by the tail cuff method in the senescent rats (130±4 mmHg) was lower than in the young rats (147±3 mmHg, $P<0.01$).

3.2. Cardiac work

In the pre-pacing period in untreated hearts, the work capacity per g wet weight of senescent hearts was only 74% of that in young hearts (9.9±0.6 vs. 13.3±1.5 mJ/g/sec, $P<0.05$). In senescent hearts CoQ$_{10}$ treatment increased cardiac work by 28% compared to untreated (12.7±0.6 vs. 9.9±0.6 mJ/g/sec; $P<0.01$) (Fig. 1). By contrast, in young hearts CoQ$_{10}$ had no effect. After pacing stress, cardiac work was markedly reduced in all four groups, regardless of age or treatment but especially in the senescent untreated group. The striking finding was that CoQ$_{10}$ treatment in senescent hearts produced a fourfold increase in post-stress work from 1.6±0.6 mJ/g/s to 6.1±0.7 mJ/g/s ($P<0.001$) (Fig. 1). This level of work capacity in CoQ$_{10}$ treated senescent hearts was just as high as that in the young hearts. CoQ$_{10}$ treatment had no effect.
on post-stress function in young hearts (Fig. 1). This age-specific augmentation in work performance by CoQ<sub>10</sub> was also clearly manifested when post-stress work was normalized in terms of pre-stress function (Fig. 2). In senescent hearts CoQ<sub>10</sub> treatment improved recovery of work from 16.8±4.3% to 48.1±4.1% (P<0.0001). By contrast in young hearts CoQ<sub>10</sub> treatment produced no change (44.5±7.4 to 42.3±7.3%).

3.3. Coronary flow

Before pacing, coronary flow was significantly lower in the senescent hearts than in the young hearts, in both the nonworking (13.3±0.7 vs. 16.9±0.9 ml/g/min: P<0.01) and working modes (12.9±0.6 vs. 18.2±0.8 ml/g/min: P<0.0001), with no significant effect of treatment in either age group (P=0.9). During pacing, coronary flow was significantly less in the senescent animals compared to the young rats (P<0.0001). In senescent hearts coronary flow was increased by treatment with CoQ<sub>10</sub> (P<0.0001).

The initiation of pacing increased coronary flow in all groups by approximately 25%. During pacing, in untreated animals coronary flow was significantly less in the senescent than in the young (P<0.0001) (Fig. 3). In senescent hearts, treatment with CoQ<sub>10</sub> was associated with increases in coronary flow (P<0.0001) to levels similar to those seen in the young hearts. There was no effect of CoQ<sub>10</sub> treatment on coronary flow in the young hearts.

After pacing in all four groups coronary flow was significantly lower than before pacing (P<0.0001). In untreated hearts post-pacing coronary flow (Fig. 3) was significantly lower in the senescent group than in the young (P<0.01). CoQ<sub>10</sub> treatment of old rats provided some benefit to post-pacing coronary flow.

3.4. MVO<sub>2</sub> and efficiency

Before pacing MVO<sub>2</sub> in untreated working senescent hearts was only 66% of that in the young hearts (174±8.4 vs. 262.8±12.8 µL/g/min: P<0.0001) (Fig. 4). However, despite its effect in enhancing work performance, CoQ<sub>10</sub> treatment did not significantly increase MVO<sub>2</sub> in the senescent age group compared to the untreated senescent group (195.1±15.1 vs. 174±8.4 µL/g/min: P=0.21) (Fig. 4). The end result of these two effects (increased work without a corresponding increase in oxygen consumption) was increased efficiency in CoQ<sub>10</sub> treated senescent hearts, 19.1±3.2% vs. untreated senescent hearts, 16.3±1.8% (P<0.05).

During pacing MVO<sub>2</sub> showed a similar response pattern to coronary flow with increases in all four groups (Fig. 4). There was an age-specific effect of CoQ<sub>10</sub> treatment on
MVO\textsubscript{2} with a significant increase in the senescent hearts, approaching levels seen in the young hearts ($P<0.0001$), but with no effect in young hearts.

After pacing there was a trend towards reduced recovery of MVO\textsubscript{2} in senescent hearts compared to young hearts ($61.3\pm4.0$ vs. $74.1\pm5.0\%$; $P=0.06$) (Fig. 2). Recovery of pre-pacing efficiency in senescent hearts was less than in young hearts ($25.7\pm6.5$ vs. $61.4\pm12.0\%$; $P<0.05$). CoQ\textsubscript{10} treatment produced significant improvements in post-stress recovery of both MVO\textsubscript{2} and efficiency in senescent hearts but had no effect in young hearts (Fig. 2).

### 3.5. Myocardial high energy phosphate levels

Measured after recovery from pacing stress, mean levels of myocardial ATP and creatine phosphate were similar in all groups and only just below normal levels for our laboratory ($23.4\pm0.8$ and $33.9\pm1.6$ $\mu$mol/g dry weight respectively). The only effect of CoQ\textsubscript{10} treatment in young and senescent rats was a small increase in ADP and AMP ($P<0.05$) (Table 1).

### 3.6. Aerobic nature of pacing stress

It was important to validate the aerobic nature of the pacing stress by demonstrating that pacing did not result in ischemic metabolism. Measurements of lactate in 25 rat hearts showed no significant difference in lactate release between age groups ($P=0.64$). Before pacing there was a small amount of lactate release in both age groups (average of two groups, $1.6\pm0.2$ $\mu$mol/g/min). With pacing, lactate release increased slightly in both age groups ($2.3\pm0.2$ $\mu$mol/g/min after 5 min and $2.8\pm0.2$ $\mu$mol/g/min after 90 min) but still remained low relative to oxygen consumption and was compatible with fully aerobic metabolism even during the period of maximal stress. Lactate release continued at a similarly low level during the post-stress working period ($2.3\pm0.3$ $\mu$mol/g/min). The mean amount of lactate released during pacing for both groups was only $2.5$ $\mu$mol/g/min which is equivalent to $5 \mu$mol of ATP produced from anaerobic metabolism. By comparison the MVO\textsubscript{2} in the senescent animals during pacing was $9.9$ $\mu$mol/g/min which is equivalent to $59 \mu$mol/g/min of ATP. In the young hearts, the mean MVO\textsubscript{2} during pacing was $12.1$ $\mu$mol/g/min, which is equivalent to $73 \mu$mol/g/min of ATP. Thus in both groups during pacing, over 91% of the energy requirements were met by oxidative metabolism, which was similar to that during the pre-stress period (95%). This result validates the aerobic nature of the pacing stress. Furthermore, myocardial lactate concentration measured at the end of the post-pacing working period was normal and did not differ between old and young age groups ($4.8\pm1.5$ vs. $3.8\pm0.4$ $\mu$mol/g dry weight respectively, $P=0.65$). No significant difference between the two age groups was observed for the lactate/pyruvate ratio ($15.7\pm2.8$ vs. $20.2\pm3.0$ respectively, $P=0.32$).

### 4. Discussion

The present study demonstrated that senescent rat hearts had reduced baseline levels of work, oxygen consumption and efficiency, and were less tolerant to pacing stress than young hearts. Second, pre-treatment of senescent animals with CoQ\textsubscript{10} improved the baseline levels of myocardial

### Table 1

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<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>CoQ\textsubscript{10}</th>
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<tbody>
<tr>
<td></td>
<td>Young (n = 5)</td>
<td>Senescent (n = 8)</td>
</tr>
<tr>
<td>ATP</td>
<td>20 ± 1.8</td>
<td>18.3 ± 1.3</td>
</tr>
<tr>
<td>ADP</td>
<td>4.2 ± 0.3</td>
<td>4.3 ± 0.3</td>
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<tr>
<td>AMP</td>
<td>0.45 ± 0.07</td>
<td>0.51 ± 0.08</td>
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<tr>
<td>TAN</td>
<td>25.0 ± 2.0</td>
<td>23.1 ± 1.6</td>
</tr>
<tr>
<td>PCr</td>
<td>38.4 ± 3.9</td>
<td>36.8 ± 4.2</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. ($\mu$mol/g dry weight); ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; TAN, total adenine nucleotides; PCr, phosphocreatine.

\* $P<0.05$, CoQ\textsubscript{10} vs. untreated in corresponding age group.
work, MVO₂ and efficiency prior to the onset of pacing and produced a marked improvement in recovery of all three functional parameters after pacing. In these terms, the performance of hearts of CoQ₁₀-treated senescent rats was restored to that of young animals. Moreover, there was no significant improvement in cardiac function of young rats treated with CoQ₁₀ compared to that of untreated young rats. We discuss below technical aspects of the study and the interpretation of our data in physiological and biochemical terms.

4.1. Experimental preparation: comparison of old and young rats

The senescent rats had hypertrophic hearts with a greater mass than the young rats. The systolic blood pressure measured in the awake state using the tail cuff method was actually lower in the senescent rats than in the young rats. Thus the mild cardiac hypertrophy in the senescent hearts was a consequence of age and not hypertension.

The pacing stress protocol used here was designed to stress mitochondria by maximizing myocardial oxygen consumption and thus oxidative phosphorylation, without producing ischemia. In order to avoid subendocardial ischemia in the pacing period, we perfused the heart in Langendorff mode at a greater pressure, (96 mmHg) than is usually used in this preparation (74 mmHg) [21]. To assess the extent of ischemia produced by pacing, we measured the release of myocardial lactate into the coronary effluent. In response to the aerobic stress the release of lactate increased minimally in both groups, thus validating the aerobic nature of the pacing stress.

Because of the slower pre-pacing native heart rate in the senescent (212±4 bpm) than in the young hearts (272±5 bpm), the increase in heart rate by pacing to the same high level (510 bpm) in both groups represented a greater proportional increase in rate in the senescent (58%) than in the young hearts (47%). This could have represented a greater level of aerobic stress in the older hearts, although as we have shown, this rate did not cause ischemia. However, in the untreated senescent hearts the standard pacing rate of 510 bpm evoked a much lower level of myocardial oxygen consumption in the senescent hearts than in the young hearts and therefore actually represented a lower level of aerobic stress in the senescent hearts (Fig. 4). Even with this lower level of stress, recovery of pump function after pacing in the untreated senescent hearts (17%) was much less than in the young hearts (45%) (Fig. 2).

4.2. Effect of CoQ₁₀ treatment on cardiac function

Since coronary flow in the treated senescent hearts was greater both during pacing and during the post-pacing period it might be postulated that the action of CoQ₁₀ in augmenting myocardial pump function was caused not by the direct action of CoQ₁₀ on the myocytes, but via a vasodilator effect of CoQ₁₀ on the coronary vasculature which improved coronary flow reserve and which itself increased myocardial contractility (Gregg effect) [28]. We believe that this is unlikely to be a major mechanism, for the following reasons: (1) The increases in coronary flow and MVO₂ observed in the hearts of CoQ₁₀-treated rats during post-pacing recovery (Figs. 3 and 4) are all explicable simply in terms of the increased demand for oxygen created by increased work output by the heart; (2) There is evidence that the direct effect of CoQ₁₀ on vascular smooth muscle is vasoconstriction rather than vasodilation [29]; (3) The enhanced pump function in the hearts of CoQ₁₀-treated animals was evident even in the pre-pacing period (Fig. 1) at a time when the coronary flow in the treated and untreated senescent hearts was very similar (Fig. 3). All these considerations strongly suggest a direct action of CoQ₁₀ on the senescent myocyte to enhance ATP production (as evidenced by increased MVO₂) which is most evident after a period of increased energy demand induced by aerobic pacing stress.

4.3. The senescent myocardium and mitochondrial decline

A by-product of the process of oxidative phosphorylation is a small but significant flow of oxygen free radicals. Most of these reactive oxygen species are scavenged by an array of endogenous antioxidant enzymes, principally superoxide dismutase, catalase, and glutathione peroxidase [30]. Nevertheless a proportion of free radicals escapes this protective process and causes injury principally in the mitochondria, the targets for damage including membrane lipids, DNA, and proteins [31]. There is evidence that the activity of endogenous oxygen free radical scavenger systems is altered in senescent tissues [32]. With increased age, the mitochondrial resting respiratory rate and the production of reactive oxygen species are significantly elevated in a number of tissues, including the heart [32]. As a consequence, despite advancing age, the intrinsic enzymic protective mechanisms against oxidative stress and the presence of endogenous antioxidant molecular species, there is an accumulation of oxidatively damaged macromolecules in the cell, and particularly in the mitochondria [31].

A key target for these reactive oxygen species is mitochondrial DNA, which contains a set of genes exclusively involved in mitochondrial energy production. During the aging process there is a progressive accumulation of mitochondrial DNA mutations which may contribute to a concomitant decrease in the capacity of senescent cells and tissues to produce energy by oxidative phosphorylation [12,14–16,18,33–35]. Unlike the nucleus, the mitochondrion has a relatively inefficient DNA repair system. Furthermore the damaged mitochondrial DNA and its resultant
inability to adequately replace damaged mitochondrially synthesised protein components of the oxidative phosphorylation chain, form the central elements for a vicious cycle of inefficient aerobic metabolism and increased free radical production [12,15,33]. Such loss of mitochondrial function would primarily affect the respiratory chain and ATP synthase, reducing the output of oxidative phosphorylation. Further detailed studies of oxidative phosphorylation function in the senescent rat heart would therefore seem warranted.

4.4. Biochemical considerations of the effects of CoQ10

We found that senescent hearts showed reduced recovery of pump function and efficiency compared to young hearts. We have shown for the first time that administration of CoQ10 to senescent rats ameliorates these functional deficits. However the mechanism of this age-specific effect is not clear. One possible explanation is that the direct action of CoQ10 as a redox substrate in the mitochondria significantly increases the energy output from oxidative phosphorylation in the myocardium by promoting coupled electron flow. This would improve the efficiency of ATP production through increased electron flow along the respiratory chain. The provision of additional CoQ10, itself a naturally occurring redox compound integral to respiratory chain function as a mobile lipophilic redox carrier, would increase the throughput of redox reactions that lead to reoxidation of NADH in mitochondria by consumption of molecular oxygen. Thus ATP production for cardiac contractile function would be increased. We measured only static levels of myocardial ATP after recovery from pacing and found essentially normal values in all groups, and no effect of CoQ10. To analyse the rate of ATP production and the stimulatory effect of CoQ10 would require detailed studies of mitochondrial bioenergetic parameters including mitochondrial respiration and the rate and efficiency of oxidative phosphorylation. Moreover, ATP or ΔGATP measurements using magnetic resonance spectroscopy would be valuable.

It has been reported that the membrane potential in mitochondria of rat hearts declines with age [20]. The membrane potential, measured by use of the indicator dye oxonol VI in isolated mitochondria, is a substantial component of the proton motive force whereby the energy from respiratory electron transport is harnessed chemiosmotically for synthesis of ATP. Moreover, reduced membrane potential in cardiac mitochondria of bioenergetically compromised rats is ameliorated by the addition of CoQ10 and some analogous quinones [20], which is further evidence of a direct effect of CoQ10 on the mitochondrial bioenergetic system. Measurement of the oxidative phosphorylation function of the hearts of senescent rats, with or without CoQ10 treatment, would provide further information on this point.

Another possible enzymatic target for CoQ10 is the plasma membrane oxidase [36] that is suggested as playing a role in the enhanced reoxidation of NADH to NAD+, particularly in cells with impaired mitochondrial respiratory chain function. Addition of exogenous CoQ10 has been shown to promote the activity of this enzyme [37,38], in catalysing the oxidation of NADH by molecular oxygen at the plasma membrane. It is pertinent to note that Lenaz et al. have reported deficits in the abundance of CoQ10 in the mitochondrial membrane as a function of increasing age in mammals [39]. Correction of this deficit may therefore account, at least in part, for the efficacy of exogenous CoQ10 in ameliorating senescent myocardial dysfunction. Finally, CoQ10 is known to have an antioxidant effect and thus could help the endogenous protective enzyme systems “mop up” excess reactive oxygen species and prevent the accumulation of damaged cellular components, leading to functional loss, which appears to accumulate throughout life [12,18,40]. But this would not be expected to be effective in the relatively short periods of the present experiments, in which CoQ10 was administered for 6 weeks prior to cardiac function tests. Furthermore, the aerobic stress of rapid pacing would be expected to generate fewer oxygen free radicals than ischemia/reperfusion stress which is the usual setting in which the cardioprotective effects of antioxidants are seen. Whilst we cannot yet eliminate the possibility of a cellular repair process which reduces the abundance of oxidatively damaged target molecules (such as proteins or lipids) during the 6 weeks of CoQ10 treatment, we suggest that antioxidant effects alone of CoQ10 do not provide a compelling explanation of its efficacy in the present study.

4.5. Potential clinical implications

As there were no detrimental effects of treatment observed in young or old animals, and intake of CoQ10 is simple, our findings in the rat suggest that this compound may be able to improve the tolerance of the human senescent myocardium to stress. Although further studies in animals are indicated, the clinical relevance of these findings for elderly patients is that CoQ10 may have a cardioprotective action in certain circumstances such as during the stress associated with myocardial infarction and cardiac surgery where increased energy demands need to be met for sustained cardiac viability.

5. Conclusion

We conclude that pre-treatment with CoQ10 improves the tolerance of the senescent myocardium to aerobic stress. The evidence presented here suggests an action at the myocyte level rather than on the coronary vasculature. We propose that the effects of CoQ10 seen here are not merely those of a free radical scavenger, but that this redox compound promotes restoration of electron flow in
dysfunctional electron transport chains within affected mitochondria, and that it may even act as a stimulant of alternative cellular redox pathways.

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