Coenzyme Q\(_{10}\) reverses mitochondrial dysfunction in atorvastatin-treated mice and increases exercise endurance

Ayako Muraki (村木 絵子), Kazutoshi Miyashita (宮下 和季), Masanori Mitsuishi (三石 正憲), Masanori Tamaki (田澤 昌憲), Kumiko Tanaka (伊藤 久美子), and Hiroshi Itoh (伊藤 裕)

Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan

Submitted 3 November 2011; accepted in final form 24 May 2012

Muraki A, Miyashita K, Mitsuishi M, Tamaki M, Tanaka K, Itoh H. Coenzyme Q\(_{10}\) reverses mitochondrial dysfunction in atorvastatin-treated mice and increases exercise endurance. J Appl Physiol 113: 479–486, 2012. First published May 31, 2012; doi:10.1152/japplphysiol.01362.2011.—Statin treatments reduce the levels of cholesterol and increase exercise endurance. Conversely, coenzyme Q10 treatment reverses statins and increases exercise endurance. The effect of statins, atorvastatin treatment, on mitochondrial function and physical performance was examined using cultured myocytes. We found that atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. The effect of two statins, atorvastatin and pravastatin, on mitochondrial function and physical performance was examined using cultured myocytes. We found that atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. Thus, atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency.

MATERIALS AND METHODS

**Materials, animals, and cell culture.** C57BL/6 mice were purchased from Charles River Laboratories (Tokyo, Japan), and C2C12 cells, which are mouse-derived cultured myocytes, from RIKEN BioResource Center (Tsukuba, Japan). A lipophobic statin, pravastatin, has been shown to decrease the levels of ubiquinone production. The coenzyme Q10 (CoQ10), which is a member of the ubiquinone family, has been shown to increase exercise endurance in association with statin treatment. Therefore, we hypothesized that statin treatment might influence physical performance through mitochondrial dysfunction. The effect of two statins, atorvastatin and pravastatin, on mitochondrial function and physical performance was examined using cultured myocytes. We found that atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. Thus, atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. A lipophobic statin, pravastatin, has been shown to decrease the levels of ubiquinone production. Thus, atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. Therefore, we hypothesized that statin treatment might influence physical performance through mitochondrial dysfunction. The effect of two statins, atorvastatin and pravastatin, on mitochondrial function and physical performance was examined using cultured myocytes. We found that atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. Thus, atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. Therefore, we hypothesized that statin treatment might influence physical performance through mitochondrial dysfunction. The effect of two statins, atorvastatin and pravastatin, on mitochondrial function and physical performance was examined using cultured myocytes. We found that atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. Thus, atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. Therefore, we hypothesized that statin treatment might influence physical performance through mitochondrial dysfunction. The effect of two statins, atorvastatin and pravastatin, on mitochondrial function and physical performance was examined using cultured myocytes. We found that atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency. Thus, atorvastatin treatment reverses mitochondrial dysfunction due to ubiquinone deficiency.
tin, and a lipophilic statin, atorvastatin, were courtesy gifts from Daiichi-Sankyo (Tokyo, Japan). PureSorb-Q40-CoQ10, a water-soluble form of CoQ10, was commercially available (P40-CoQ10; Nisshin Pharma Tokyo, Japan) (28, 32). C2C12 cells were grown to a nearly confluent number in Dulbecco’s modified Eagle’s medium (DMEM no. 11995; Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS), and the cells were differentiated into myocytes by incubation with 0.1% FBS for 7 days before the experiments.

**Experiments with statin-treated mice.** Male C57BL/6 mice fed normal chow (NC; 10 kcal% fat, 20 kcal% protein, and 70 kcal% carbohydrate; Research Diets, New Brunswick, NJ) were treated with pravastatin (1.0 g/kg pellet), atorvastatin (0.1 g/kg pellet), or CoQ10 (10 mg/l in drinking water) from 8 wk of age. The mice were examined for grip strength and running distance at 16 wk of age, except in the experiment shown in Fig. 4, in which examinations at 8, 10, and 12 wk of age were added to assess the earlier effect of statin and CoQ10. The mice were killed, blood samples were collected, and the tissues were harvested at 20 wk of age. Statin treatment had been continued until the mice were killed. Eight mice in each treatment group were used for the experiments. The group of each statin has been reported to achieve the maximum concentration (C_{max}) of therapeutic doses used in humans (pravastatin, 3 × 10^{-9} mol/l; atorvastatin, 3 × 10^{-9} mol/l) (5, 10a, 18). The dose of CoQ10 was reported to achieve sixfold higher serum concentration of ubiquinone in mice, which replicated a therapeutic dose in human (28).

Grip strength of the forelimbs was measured using a standard dynamometer for mice (MK-380M, Muromachi kikai, Tokyo, Japan). A mouse was put on a metal mesh and pulled horizontally. The power of traction when the mouse released the mesh was defined as the grip strength. Running distance was measured on a mouse treadmill (LE8170M, PanLab, Barcelona, Spain), according to a previously described protocol (2). For measurements of running distance, mice were forced to run on the motor-driven treadmill until they were completely exhausted, which was defined as the point at which they remained on the electrical shocker plate for more than 30 s. The treadmill was set at a constant 10% incline; the speed was 18 cm/s at the beginning and was increased by 3 cm/s every 2 min. The average time until exhaustion for the wild-type mice at 16 wk of age fed normal chow was 24 min (corresponding to 450 m of running). For measurement of blood lactate levels, after an 8-h fast and after exercise, the mice were subjected to mild exercise. The treadmill was set at a constant speed of 20 cm/s, and the mice were forced to run for 15 min. All mice could complete the running task without severe exhaustion. Whole blood lactate levels were measured before and immediately after 15 min of the mild treadmill exercise at the constant speed of 20 cm/s, and after a further 15 min of rest, using a portable lactate analyzer (Lactate-Pro, ARKRAY, Kyoto, Japan).

Respiratory gas analysis (ARCO-2000 mass spectrometer, ARCO system, Kashiwa, Japan) was performed and blood samples were collected from the inferior vena cava to determine serum cholesterol, ubiquinone, and creatine kinase levels (24). The ubiquinone level was determined by high-performance liquid chromatography (HPLC) (Nikken SEIL, Fukuroi, Japan). Tissue samples from gastrocnemius and quadriceps were obtained for histological, physiological, and molecular biological analysis. Gastrocnemius muscle was used for histological analysis and quadriceps for mitochondrial analysis, ubiquinone content, real-time PCR, and Western blotting. The muscle fiber diameter and fiber types (I, IIa, or IIb) of gastrocnemius in statin-treated mice were determined by succinate dehydrogenase (SDH) staining, which indicates fiber type by the density of staining. The fiber types were determined by the density digitized by gray-scale images of the tissue sections (15, 27). The ubiquinone and lactate levels of the tissue extracts were measured in a manner identical to those of blood. Metabolome analysis was performed using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) (Human Metabolome Technologies, Tsururoka, Japan) (17). Representative 54 energy-associated metabolites, including the intermediates of the glycolytic system and TCA cycle, were analyzed in quadriceps of atorvastatin-treated mice and the control, and 29 metabolites were successfully quantified.

All animal experiments were approved by the institutional ethic committee (the laboratory animal care and use committee of the Keio University) and were in accordance with domestic law on the protection of laboratory animals, which is based on the Declaration of Helsinki.

**Experiments using statin-treated C2C12 cultured myocytes.** C2C12 cells were grown and differentiated in 96-well assay plates and treated with pravastatin or atorvastatin (pravastatin, 10^{-7} mol/l; atorvastatin, 10^{-8} mol/l, unless otherwise indicated) with or without P40-CoQ10 at a standard dose (1 mg/l for cultured myocytes) for 48 h. The cells were examined for oxygen utilization, mitochondrial density, mitochondrial reactive oxygen species (ROS) production, and cell survival.

 Oxygen utilization of C2C12 cells was measured using a BD Oxxygen Biosensor plate (no. 353830, Becton, Dickinson and Company Biosciences, Bedford, MA), in which a fluorescent agent that emits in relation to oxygen utilization is embedded in the 96-well format plate. The presence of oxygen in the plate suppresses the fluorescent signal, and the signal increases as oxygen level decreases through cellular respiration. Cells were treated with a glucose-free galactose medium (no. 20383 L15 medium, Gibco) for 24 h before the measurement. The number of each cell group was counted using an automated counter (no. C10227 Countess, Invitrogen, Tokyo, Japan) according to the manufacturer’s instructions. We applied 4 × 10^5 cells to each well of the plate. The fluorescent signal (excitement: 485 nm; emission: 630 nm) was read for 90 min using a microplate reader (Synergy4, BioTek Instruments, Winooski, VT).

Mitochondrial density and mitochondrial ROS production of C2C12 cells were determined by use of fluorescent dyes, MitoTracker Green FM and MitoSOX Red (Molecular Probes, Eugene, OR), as described previously (25). C2C12 cells (sparse dissemination at 10^5 cells/cm^2) were visualized by means of a confocal microscope (LSM510; Carl Zeiss, Tokyo, Japan) with staining by the fluorescent probes. Images were acquired with a ×200 magnification. Cell survival was determined using a commercially available assessment kit that distinguishes live from dead cells (no. 1.3224 LIVE/DEAD Viability/Cytotoxicity assessment kit, Invitrogen, Carlsbad, CA). The number of living C2C12 cells after the treatments was measured, and the cell survival was estimated.

**Isolation of mitochondria and evaluation of mitochondrial activity.** Mitochondria were isolated from the tissues or cultured cells using a commercially available kit (KC010100, BioChain Institute, Hayward, CA), according to the manufacturer’s instructions. Activity of the electron transporter complex I (NADH oxidase) was quantified on the basis of the consumption rate of nicotinamide adenine dinucleotide (NADH), as previously described (9), and defined as mitochondrial activity. Isolated mitochondria were mixed with NADH to initiate the reaction and reach a final concentration of 1 mmol/l NADH. NADH consumption was monitored by the absorbance value at 340 nm using a spectrophotometer (Nanodrop 1,000, Thermo Fisher Scientific, Wilmington, DE). A decrease in NADH levels reflects the mitochon-
drial activity. Mitochondrial content was determined by mitochondrial DNA copy number, which was defined as the amount of mitochondrial genome relative to nuclear genome, estimated using quantitative polymerase chain reaction (PCR) (25).

**Estimation of gene expression and protein levels by real time PCR and western blotting.** Real-time PCR and Western blotting were performed by standard methods to evaluate the expression and protein levels of molecules participating in energy metabolism in the skeletal muscles of statin-treated mice. Total RNA was extracted with the help of an RNeasy Mini kit (Qiagen, Tokyo, Japan), and reverse transcription was performed with an ExScript RT reagent kit (TaKaRa Bio, Otsu, Japan). Gene expression levels were determined by quantitative PCR reactions (ABI 7500) in the presence of primer pairs of the SYBR Premix Ex Taq (TaKaRa Bio) fluorescent dye. The primer sequences for real-time PCR were as follows;
peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α): forward CGTAAATCTGCGGATGATG, reverse CAGTTTCGTCGACCTGCTGA; mitochondrial transcription factor A (Tfam): forward TGAAGCTTGAATAGGGCTTGA, reverse CGGATGTTCACACTTCGAC.

Total protein extracts (10 μg) from gastrocnemius were separated by polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes using a dry blotting system (iBlot, no. IB1001, Invitrogen), and incubated with the antibodies. Immunolabeled proteins were detected using a chemiluminescence kit (ECL Plus; GE Healthcare, Piscataway, NJ) and a Lumino-image analyzer (LAS-3000; FujiFilm, Tokyo, Japan). The density of the blot for each protein relative to that for the internal control, β-actin, was estimated by imaging software (MultiGauge; FujiFilm).

The primary antibodies used for Western blotting were as follows: porin (1:2,000; A21317, Molecular Probes, Eugene, OR), ATP synthase (1:2,000; A21350, Molecular Probes), pyruvate dehydrogenase kinase 1 [PDHK1 (1:1,000; no. 3820, Cell Signaling, Danvers, MA)], PDHK4 (1:1,000; LS-B3459, LifeSpan Biosciences, Seattle, WA), and β-actin (1:2,000; no. 4967, Cell Signaling).

Statistical analysis. All data were expressed as means ± SE. Comparison of means between the two groups was performed by the Student’s t-test. When more than two groups were compared, analysis of variance was used to evaluate significant differences among groups, and if significant differences were confirmed, each difference was further examined by Fisher’s protected least significant difference method. P < 0.05 was considered to be statistically significant.

RESULTS

Atorvastatin decreases exercise endurance in mice without change in muscle mass. To examine the effect of statins on physical performance, male C57BL/6 mice were treated with either pravastatin (1.0 g/kg diet, a water-soluble statin) or atorvastatin (0.1 g/kg diet, a lipid-soluble statin) from the age of 8 wk old and were examined for grip strength and running distance up to 16 wk. Mice were assigned to the following three treatment groups: control, pravastatin, and atorvastatin (n = 8 mice in each treatment group). Running distance of atorvastatin-treated C57BL/6 mice decreased significantly compared with the control (18.3% decrease compared with control, P < 0.01, Fig. 1A). In contrast, pravastatin-treated mice did not show the decrease. Grip strength of the statin-treated mice was similar to the control (Fig. 1B). There was no significant difference in the weight of gastrocnemius muscle among the three groups (Fig. 1C), and serum creatine kinase (CK) levels were similar (Fig. 1D). However, the blood lactate levels of atorvastatin-treated mice showed a significant increase after treadmill exercise, which was measured immediately after 15 min of exercise and after a further 15 min rest following the exercise (Fig. 1E). Pravastatin-treated mice did not show a similar increase in lactate level before or after the exercise. Mitochondrial activity in the quadriceps of atorvastatin-treated mice decreased significantly compared with that of the control or pravastatin group (22.2% decrease compared with control, P < 0.01, Fig. 1F), and ubiquinone content in the quadriceps of atorvastatin-treated mice also decreased significantly (Fig. 1G). The muscle fiber types were not changed by the statin-treatment (data not shown).

Atorvastatin decreases mitochondrial activity and oxygen utilization through reduction in ubiquinone content in cultured myocytes. To further explore effects of the statins on energy metabolism, C2C12 cultured myocytes were treated with pravastatin or atorvastatin for 48 h, and oxygen utilization, mitochondrial activity, and cell viability were examined. Atorvastatin at a lower concentration (10−8 mol/l, which is similar to Cmax of a therapeutic dose of atorvastatin in humans), significantly decreased oxygen utilization in the cultured myocytes (n = 4 independent experiments, P < 0.01 vs. controls, Fig. 2A) in association with a decrease in mitochondrial activity (Fig. 2B), without affecting the cell number (Fig. 2C, at 10−8 mol/l). Atorvastatin at 10 times higher concentration (10−7 mol/l, which is similar to Cmax of a therapeutic dose of pravastatin in humans), did not change oxygen utilization and mitochondrial activity (Fig. 2A, B and C). The number of living C2C12 cells after they were treated with each statin for 48 h in the range of 10−8 to 10−5 mol/l was measured, and the cell survival was estimated. Atorvastatin caused a significant decrease in the cell number at a concentration higher than 10−7 mol/l in a dose-dependent manner, and the number prominently decreased after the treatment with a higher concentration of atorvastatin (Fig. 2C). However, pravastatin did not significantly alter the number until a concentration of 10−6 mol/l (Fig. 2C). Lactate level in the culture medium of atorvastatin-treated C2C12 cells (10−8 mol/l) increased (Fig. 2D), while the ubiquinone content in the cell decreased (Fig. 2E). In contrast, pravastatin at 10−7 mol/l did not cause a significant change in either lactate or ubiquinone level in the cultured myocytes (Fig. 2, D and E). Mitochondrial density and mitochondrial ROS production of the C2C12 cells showed no significant differences among the experimental groups after treatment with each statin (Fig. 2F). The microscopic images, which were obtained with fluorescent probes for mitochondria and a confocal microscope, showed no significant differences among the groups (Fig. 2G).

CoQ10 increases mitochondrial activity and oxygen utilization in atorvastatin-treated cultured myocytes. As mentioned above, atorvastatin decreased oxygen utilization in muscle cells in association with decreased ubiquinone level and mitochondrial activity. Therefore, we examined the effect of one type of ubiquinone,
CoQ10, on energy metabolism of atorvastatin-treated C2C12 myocytes. A water-soluble form of CoQ10 was added to the culture medium at a standard dose (1 mg/l) used in previous reports (19, 30). The oxygen utilization and mitochondrial activity of C2C12 cells significantly decreased after treatment with 10<sup>-8</sup> mol/l atorvastatin. Both decreases were completely reversed after CoQ10 treatment (*n* = 4 independent experiments, *P* < 0.05, Fig. 3, A and B). Atorvastatin-related decrease in cell viability was also reversed by CoQ10 (†*P* < 0.05, ††*P* < 0.01 vs. control, Fig. 3, C).
significantly suppressed after CoQ10 treatment (Fig. 3C). Atorvastatin-related increase in lactate levels was completely eliminated after CoQ10 treatment (Fig. 3D). CoQ10 treatment alone without atorvastatin did not change oxygen utilization, mitochondrial activity, or lactate production in C2C12 cells (Fig. 3, A, B, and D). Mitochondrial density and mitochondrial ROS production showed no significant differences among the experimental groups at 48 h after CoQ10 treatment with or without atorvastatin (Fig. 3E).

**CoQ10 increases exercise endurance in atorvastatin-treated mice through enhancement of mitochondrial activity in muscle.** The effect of CoQ10 in atorvastatin-treated C57BL/6 mice was examined by adding a water-soluble form of coenzyme Q10 to drinking water (10 mg/l). Mice were assigned to the following four treatment groups: control, CoQ10 alone, atorvastatin, and atorvastatin plus CoQ10 (n = 8 mice in each treatment group). Running distance significantly decreased in atorvastatin-treated mice after 4 wk of treatment, and in contrast, CoQ10 recovered the decrease in exercise endurance (Fig. 4A). CoQ10 treatment caused a 13.8% increase in running distance of the atorvastatin-treated mice at 16 wk of age (n = 8, P < 0.01, Fig. 4A). Conversely, CoQ10 treatment alone did not improve exercise endurance or grip strength (Fig. 4, A and B). The grip strength showed no significant difference when mice were treated with either atorvastatin or CoQ10 (Fig. 4B). Serum ubiquinone levels, which were equivalent between atorvastatin-treated mice and the control, increased to a sixfold higher level by CoQ10 treatment (Fig. 4C). The significant decrease in

---

**Fig. 4. CoQ10 increases exercise endurance in atorvastatin-treated mice through enhancement of mitochondrial activity in muscle.** C57BL/6 mice were assigned into 4 groups: control, CoQ10 (10 mg/l in drinking water), atorvastatin (0.1 g/kg diet), or atorvastatin + CoQ10 (n = 8 in each treatment group). *P < 0.05, **P < 0.01 vs. control; †P < 0.05 vs. atorvastatin, ††P < 0.01 vs. atorvastatin. A: running distance of CoQ10-treated mice with or without atorvastatin. B: grip strength of forelimbs. C: ubiquinone levels in serum and quadriceps. D: mitochondrial activity of isolated mitochondria from quadriceps of CoQ10-treated mice with or without atorvastatin. E: mitochondrial activity from quadriceps of CoQ10-treated mice with or without atorvastatin. F: blood lactate levels of CoQ10-treated mice with or without atorvastatin measured before and after exercise. F: oxygen utilization of CoQ10-treated mice, examined by expiratory gas analysis during night (2000–0800) and day (0800–2000). G: mitochondrial content in the quadriceps determined by mitochondrial DNA copy number. H: quantitative PCR analysis of expression of genes involved in mitochondrial biogenesis: PGC1α, PPAR gamma co-activator 1α, TFam, mitochondrial transcription factor A. I and J: Western blot analysis of the protein levels of porin, ATP synthase (ATPsyn), pyruvate dehydrogenase kinase 1 (PDHK1), and PDHK4 in the quadriceps of CoQ10-treated mice with or without atorvastatin. Images of the blots (I) and the result of densitometry (J) are shown.
ubiquinone level and mitochondrial activity in quadriceps of atorvastatin-treated mice recovered when CoQ10 was coadministrated with atorvastatin (Fig. 4G, C and D). Conversely, the increase in blood lactate level after exercise in atorvastatin-treated mice was significantly decreased by CoQ10 treatment (Fig. 4E). Oxygen utilization in atorvastatin-treated mice, which was estimated by indirect calorimetry, was found to decrease during the day time; however, it recovered after CoQ10 treatment (Fig. 4F). The mitochondrial content in quadriceps, which was estimated by the mitochondrial DNA copy number, exhibited no significant difference when mice were treated with atorvastatin or CoQ10 (Fig. 4G). Expression of genes involved in mitochondrial biogenesis, such as PGC1α and Tfam, was similar between the experimental groups (Fig. 4H). The protein levels of molecules within the mitochondria, such as porin and ATP synthase, also showed no significant difference among the groups (Fig. 4, I and J). On the other hand, PDHK1 and -4, which reduce aerobic glycolysis by suppressing pyruvate dehydrogenase (PDH) when mitochondrial dysfunction occurs and contribute to decreased exercise endurance (1, 10, 20), showed significant increases after atorvastatin treatment (26% and 19% increase for PDHK1 and -4, respectively, n = 8, P < 0.01, Fig. 4J). The increase in PDHKs was reversed by CoQ10 treatment (Fig. 4J).

Through these experiments, we elucidated that CoQ10 treatment reverses the atorvastatin-related decrease in exercise endurance in mice by abrogating ubiquinone deficiency, and thus recovering mitochondrial activity and oxygen utilization (Fig. 5).

DISCUSSION

The present study demonstrated that atorvastatin induced muscular mitochondrial dysfunction due to a ubiquinone deficiency in mice, thereby decreasing exercise endurance. Meanwhile, muscle mass, strength, and fiber composition were not affected by the same dose of atorvastatin. CoQ10 could reverse atorvastatin-related decreases in mitochondrial function and exercise endurance due to muscular ubiquinone deficiency. The findings suggest that ubiquinone supplementation by means of water-soluble CoQ10 in addition to statin therapy is useful for preventing statin-related decreases in exercise endurance, particularly when a lipophilic statin is used. In previous reports, ubiquinone deficiency in statin-treated patients was demonstrated (21), and ubiquinone supplement by CoQ10 was effective to reduce some symptoms of statin-related myopathies (7). However, a decrease in exercise endurance has not been highlighted as a symptom of statin-related myopathy, and mitochondrial dysfunction has not been elucidated as a mechanism by which statins affect physical performance. In the present study, we found that atorvastatin developed muscular mitochondrial dysfunction due to local ubiquinone deficiency, thereby causing a decrease in exercise endurance, although muscle mass, strength, fiber composition, and serum creatine kinase level were not altered. Therefore, the decrease in exercise endurance caused by muscle ubiquinone deficiency was indicated to be an early-stage manifestation of atorvastatin-related myopathy. In addition, treatment with water-soluble CoQ10 reversed the atorvastatin-related mitochondrial dysfunction and exercise intolerance through enhancement of muscular ubiquinone level.

We found that the two statins act differently on muscular ubiquinone content; that is, atorvastatin treatment at the dose used caused a significant decrease in muscular ubiquinone in mice, but meanwhile pravastatin did not. Ubiquinone in the skeletal muscle has been reported to be synthesized locally through a HMG-CoA reductase-dependent pathway under normal conditions and not to rely on an exogenous supply (4), although other tissue might depend on it. Therefore, atorvastatin was supposed to decrease muscular ubiquinone level by suppressing muscular HMG-CoA reductase, although pravastatin did not affect it. We believe that the different membrane permeability between the statins may explain the different effect on muscular ubiquinone level (16).

We administered statins to mice (pravastatin, 1.0 g/kg diet; atorvastatin, 0.1 g/kg diet) and cultured myocytes (pravastatin, 10−7 mol/l; atorvastatin, 10−8 mol/l) as to achieve the Cmax of clinical use in humans (pravastatin, 3 × 10−8 mol/l; atorvastatin, 3 × 10−9 mol/l) (32). We adopted 10 times higher doses of pravastatin compared with atorvastatin because, in clinical use, daily pharmacological dose of pravastatin (40–80 mg/day) is 4–8 times higher than that of atorvastatin (10–20 mg/day). Therefore, the doses of statins for mice and cells in this study were equivalent to pharmacological use. The dose of CoQ10 provided in the drinking water (10 mg/l) replicated a previous report in rodents and a therapeutic dose in human (28).

CoQ10 supplementation in atorvastatin-treated mice recovered muscular mitochondrial activity and exercise endurance accompanied with a decrease in postexercise lactate production. Conversely, treatment with CoQ10 alone did not change either oxygen utilization or running distance. These results indicate that exogenous administration of CoQ10 could increase muscular ubiquinone only when it was reduced from physiological levels and the administration would not increase muscular ubiquinone under normal conditions, probably because the endogenous production is sufficient enough. The notion is compatible with a previous report, which has demonstrated that CoQ10 supplementation would be effective for improving statin-related myopathies due to ubiquinone deficiency (7); however, it would not be so beneficial for improving exercise endurance in healthy individuals (26).

In summary, the findings of the present study provide the scientific and mechanistic basis for CoQ10 supplementation to statin users; that is, statin use could cause muscular ubiquinone

**Fig. 5. Suggested effect of CoQ10 on a decrease in exercise endurance of atorvastatin-treated mice.** The present study revealed that CoQ10 could reverse atorvastatin-related decreases in mitochondrial function and exercise endurance due to muscular ubiquinone deficiency.
deficiency and mitochondrial dysfunction, which would result in a decrease in exercise endurance. The decrease in endurance was identified as an early-stage manifestation of statin-related myopathy in mice and was shown to be treatable by CoQ10. The results address the need for further elucidation of the role for mitochondrial function in other manifestations of statin-related myopathy and the effect of CoQ10 supplementation to statin users, for which clinical evidence is scarce (22).

REFERENCES


