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Effect of Coenzyme Q_{10} Supplementation in Statin-Treated Obese Rats

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Abstract

Statins, HMG-CoA reductase inhibitors, are known to cause serious muscle injuries (e.g. myopathy, myositis and rhabdomyolysis), and these adverse effects can be rescued by co-administration of coenzyme Q_{10} (CoQ_{10}) with statins. The goal of the current research is to assess the efficacy of combined treatment of CoQ_{10} with Atorvastatin for hyperlipidemia induced by high-fat diet in SD rats. 4-week-old Sprague-Dawley male rats were fed normal diet or high-fat diet for 6 weeks. Then, rats were treated with either Statin or Statin with various dosages of CoQ_{10} (30, 90 or 270 mg/kg/day, p.o.) for another 6 weeks. Compared to Statin only-treatment, CoQ_{10} supplementation significantly reduced creatine kinase and aspartate aminotransferase levels in serum which are markers for myopathy. Moreover, CoQ_{10} supplementation with Statin further reduced total fat, triglycerides, total cholesterol, and low-density lipoprotein-cholesterol. In contrast, the levels of high-density lipoprotein-cholesterol and CoQ_{10} were increased in the CoQ_{10} co-treated group. These results indicate that CoQ_{10} treatment not only reduces the side effects of Statin, but also has an anti-obesity effect. Therefore an intake of supplementary CoQ_{10} is helpful for solving problem of obese metabolism, so the multiple prescription of CoQ_{10} makes us think a possibility that can be solved in being contiguous to the obesity problem, a sort of disease of the obese metabolism.

Key Words: Statins, Coenzyme Q_{10}, Hyperlipidemia, HMG-CoA reductase, Myopathy

INTRODUCTION

Statins are widely used for the treatment of hypercholesterolemia and for the prevention of cardiovascular diseases. These drugs inhibit the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver (Alberts *et al*., 1980; Stancu and Sima, 2001). Reduction of intracellular cholesterol induces the activation of sterol regulatory element binding proteins (SREBPs) which activate the gene expression of low-density lipoprotein (LDL) receptor, resulting in the reduction of circulating LDL (Sehayek *et al*., 1994; Stancu and Sima, 2001).

Statins are the most efficient drugs for reducing plasma cholesterol level, and generally well-tolerated (Golomb and Evans, 2008). The most common adverse effects of statins are liver and muscle damage including elevated liver enzyme levels in serum, myopathy, myositis and rhabdomyolysis (Manoukian *et al*., 1990; Nakahara *et al*., 1998; Delbosc *et al*., 2002). As a result of a common biosynthesis pathway, both cholesterol and Coenzyme Q_{10} (CoQ_{10}) biosynthesis are decreased by statin treatment (Diebold *et al*., 1994; Nakahara *et al*., 1998; Satoh and Ichihara, 2000; Berthold *et al*., 2006).

CoQ_{10} (also known as Ubiquinone) is a water insoluble component of virtually all cell membranes, and has multiple metabolic functions (Quinzii *et al*., 2007). It is a key component of the mitochondrial electron transport system (Crane, 2001; Littarru and Langsjoen, 2007). Therefore, CoQ_{10} deficiency resulting from statin treatment may impair cellular energy metabolism, and contribute to the development of myopathy and muscle symptoms, as described in patients treated with statins (Franc *et al*., 2003; Thompson *et al*., 2003; Zita *et al*., 2003).

In the clinical study by Thibault (Thibault *et al*., 1996), CoQ_{10} supplementation significantly reduced the severity of statin-induced myopathy. Later, Kim *et al*. reported that the elevated serum creatine kinase levels in two lovastatin-treated patients with mild myalgia and muscle weakness were completely reversed by CoQ_{10} supplementation (Kim *et al*., 2001). Most recently, a clinical study with thirty-two patients (15 women, 7 men) treated for hyperlipidemia with statin showed that CoQ_{10}

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Table 1. Effect of CoQ_{10} supplementation on liver and heart weight in the Statin-treated obese rats

Group	Liver		Heart	
	Total	Relative	Total	Relative
N	9.58 ± 0.22$^{\#\#}$	2.21 ± 0.02	1.32 ± 0.02$^{\#\#\#\$\$}$	0.30 ± 0.00
NSC270	9.54 ± 0.29$^{\#\#}$	2.29 ± 0.03	1.26 ± 0.02$^{\#\#\#\$\$\$}$	0.30 ± 0.00
H	11.00 ± 0.56**	2.30 ± 0.08	1.51 ± 0.02***$^{\$}$	0.32 ± 0.00$^{\$}$
HS	10.42 ± 0.22	2.20 ± 0.04	1.42 ± 0.03**$^{\#}$	0.30 ± 0.01$^{\#}$
HC	9.62 ± 0.11$^{\#\#}$	2.07 ± 0.03$^{\#}$	1.42 ± 0.03**$^{\#}$	0.31 ± 0.01
HSC30	10.43 ± 0.18	2.26 ± 0.03	1.44 ± 0.01***	0.31 ± 0.00
HSC90	10.07 ± 0.26	2.18 ± 0.04	1.42 ± 0.01*$^{\#}$	0.31 ± 0.00
HSC270	9.55 ± 0.25$^{\#\#}$	2.19 ± 0.05	1.34 ± 0.03$^{\#\#\#}$	0.31 ± 0.01

Values are means ± S.E. (n=10). *p<0.05, **p<0.01, ***p<0.001 vs. normal diet group, $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 vs. high-fat diet group, $^{\$}p$<0.05, $^{\$\$}p$<0.01, $^{\$\$\$}p$<0.001 vs. high-fat diet with Statin group. N: normal diet group, NSC270: normal diet with Statin and CoQ_{10} (270 mg/kg) group, H: high-fat diet group, HS: high-fat diet with Statin group, HC: high-fat diet with CoQ_{10} (270 mg/kg) group, and HSC30, HSC90, HSC270: high-fat diet with Statin and CoQ_{10} at various doses (30, 90 or 270 mg/kg) group.

supplementation may decrease myopathic symptoms caused by statin treatment (Caso *et al*., 2007).

In the present study, we investigated the effect of CoQ_{10} supplementation on the adverse effect induced by Atorvastatin (Statin) treatment in Sprague-Dawley (SD) rat. As an indicator for muscle damage, aspartate aminotransferase (AST), alanin aminotransferase (ALT) and creatine kinase levels in serums were monitored (Vanholder *et al*., 2000; Huerta-Alardin *et al*., 2005; Bosch *et al*., 2009). Histological analysis was performed to examine the effect of CoQ_{10} on rhabdomyolysis. In addition, to test the effect of CoQ_{10} on hyperlipidemia, total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) levels in serums were measured after 6 weeks of Satin and/or CoQ_{10} treatment in obese rats.

MATERIALS AND METHODS

Animals and experimental diets

4-week-old male SD rats (Koatech, Pyeongtaek, Korea) were housed in a temperature (23 ± 3°C) and humidity (55 ± 15%) controlled room with a ratio of 12-hour light/12-hour darkness, and were fed normal diet (Jongang Lab Animal, Seoul, Korea) for 1 week. Then, the animals were separately fed two kinds of diets, the normal diet and the high-fat diet for 6 weeks. The compositions of the normal diet and the high-fat diet which modified the AIN-76 dietary composition (Reeves *et al*., 1993), are shown in Supplemental Table 1.

Drugs, dosages and route of administration

After 6 week diet, the animals were subdivided into eight groups (Supplemental Table 2); normal diet group (N), normal diet with Statin and CoQ_{10} (270 mg/kg) group (NSC270), high-fat diet group (H), high-fat diet with Statin group (HS), high-fat diet with CoQ_{10} (270 mg/kg) group (HC), and high-fat diet with Statin and CoQ_{10} at various doses (30, 90 or 270 mg/kg) group (HSC30, HSC90, HSC270). Coenzyme Q_{10} (CoQ_{10}) and Statin (Atorvastatin calcium) were kindly provided by Daewoong Pharmaceutical Co., Ltd. (Seoul, Korea). Rats were administered with Statin and/or CoQ_{10} orally once a day for 6 weeks. Body weights and food efficiency ratio (FER) were monitored twice a week. FER was determined as follows: FER=weight gain per day (g/day)/food intake per day (g/day). At the end of the treatment, rats were anesthetized with CO_2 gas. The epididymal, abdominal, visceral fat-pads, livers and hearts were surgically removed, weighed, snap-frozen in liquid nitrogen, and stored at -70°C until use. This study was approved by the Institutional Animal Care and Use Committee of Kyung Hee University, Seoul, Korea.

Table 2. Effect of CoQ_{10} supplementation on serum ubiquinone and creatine kinase levels in Statin-treated obese rats

Group	Ubiquinone (ng/ml)	Creatine kinase (U/L)
N	<20.00	400.10 ± 43.57$^{\$\$}$
NSC270	71.00 ± 5.46	410.90 ± 39.13$^{\$\$}$
H	<20.00	554.60 ± 31.51
HS	<20.00	652.40 ± 36.40**
HC	148.90 ± 13.32	529.20 ± 59.65
HSC30	35.33 ± 2.98	593.20 ± 46.04**
HSC90	60.89 ± 4.32	570.70 ± 50.34
HSC270	85.11 ± 6.50	462.56 ± 52.38$^{\$\$}$

Values are means ± S.E. (n=10). **p<0.01 vs. normal diet group, $^{\$\$}p$<0.01 vs. high-fat diet with Statin group. N: normal diet group, NSC270: normal diet with Statin and CoQ_{10} (270 mg/kg) group, H: high-fat diet group, HS: high-fat diet with Statin group, HC: high-fat diet with CoQ_{10} (270 mg/kg) group, and HSC30, HSC90, HSC270: high-fat diet with Statin and CoQ_{10} at various doses (30, 90 or 270 mg/kg) group.

Biochemical analysis

Blood samples were collected from the hepatic portal vein using vacutainer (Becton Dickinson&Co. Franklin Lakes, USA). Serums were separated by centrifugation at 3,000 × rpm for 15 min at 4°C. TC, HDL-C, LDL-C, TG, creatine kinase, ALT, and AST levels in serums were determined enzymatically using commercial kits (Asan Diagnostics, Seoul, Korea).

Determination of serum ubiquinone (Coenzyme Q_{10})

Serum proteins were precipitated with ethanol. The liquid phases were extracted three times with n-hexane and the organic phases were pooled and evaporated. The extract was

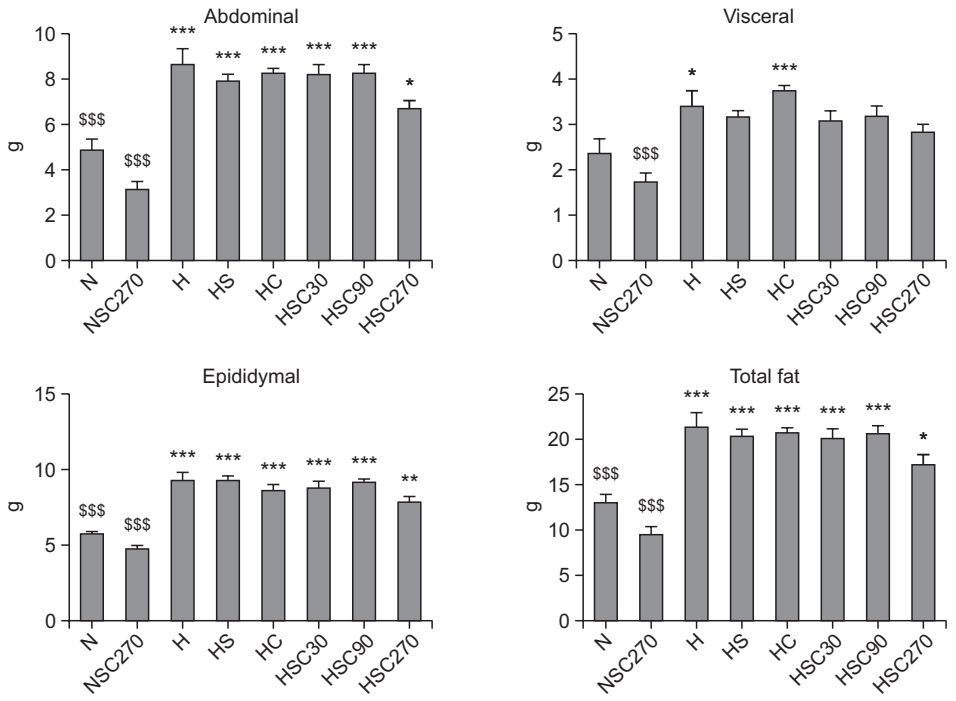

Fig. 1. Effect of CoQ_{10} supplementation on adipose tissue weight in Statin-treated obese rats. Data are mean ± S.E. (N=10). *p<0.05, **p<0.01, ***p<0.001 vs. normal diet group, $^{\$\$\$}p$<0.001 vs. high-fat diet with Statin group. N: normal diet group, NSC270: normal diet with Statin and CoQ_{10} (270 mg/kg) group, H: high-fat diet group, HS: high-fat diet with Statin group, HC: high-fat diet with CoQ_{10} (270 mg/kg) group, and HSC30, HSC90, HSC270: high-fat diet with Statin and CoQ_{10} at various doses (30, 90 or 270 mg/kg) group.

reconstituted with 15:85 dichloromethane-methanol mixtures and analyzed by high-performance liquid chromatography (HPLC). Total concentrations were determined by the area under the curve of external standard at 270 nm.

Heart histology

The heart tissues were fixed in 10% formalin and embedded in paraffin wax. Frozen heart sections from fixed tissues were cut in 10 μm thick and mounted on the slide glasses. To detect lipids, the sections were immersed in propylene glycol for 5 min and stained with Oil red O (Sigma, St. Louis, MO, USA) for 7 min. After rinsing with 85% propylene glycol and water, tissue samples were counterstained with Mayer's haematoxylin for microscopic examination.

Statistical analysis

Statistical analysis was performed by One-Way ANOVA (SPSS version 13.0; SPSS, Inc., Chicago, USA). Differences between groups were considered statistically significant at p< 0.05. Data are presented as mean ± S.E. values.

RESULTS

Body weight, lipid weight and food efficiency ratio (FER)

The high-fat diet group (H) showed significantly higher body weights and FER than the normal diet group (N). Statins-treated high-fat diet group (HS) showed a similar increase of body weight as the high-fat diet group (H). However, the body weight of the CoQ_{10} (270 mg/kg) treated group (HSC270) was significantly lower compared to that of the non-treated group (HS) (Supplemental Table 3).

Total fat was dramatically increased by high-fat diet (N: 12.81 ± 1.07, H: 21.22 ± 1.58 g) and this increase was slightly attenuated by Statin treatment (HS: 20.20 ± 0.78 g) (Fig. 1). Interestingly, CoQ_{10} treatment also reduced the increase of total fat induced by high-fat diet (HC: 20.48 ± 0.76 g), and the combined treatment of Statin and CoQ_{10} significantly lowered total fat weight (HSC270: 17.14 ± 1.02 g). In addition, the abdominal and epididymal fat weights in the HSC270 group were also significantly reduced by CoQ_{10} supplementation (Fig. 1).

Effect of CoQ_{10} supplementation on liver and heart weight in Statin-treated rats

The influence of CoQ_{10} administration on liver and heart weight in Statin-treated rats were investigated (Table 1). Compared to the normal diet group (N: 9.58 ± 0.22 g), the high-fat diet group (H: 11.00 ± 0.56 g) showed significant increase of liver weight (p<0.01), suggesting high-fat diet-induced obesity in rats as expected. The liver weight of the Statin-treated group in high-fat diet (HS) was marginally lower than that of the non-treated group (H). In contrast, compared to the high-fat diet group (H), CoQ_{10} treatment in the high-fat diet group (HC) showed significant decrease of liver weight (9.62 ± 0.11 g, p<0.01). In addition, co-administration of CoQ_{10} with Statin in the high-fat diet group (HSC) decreased liver weight in a dose-dependent manner. Heart weight of the normal diet group (N: 1.32 ± 0.02 g) was significantly lower than that of the high-fat diet group (H: 1.51 ± 0.02 g). Either Statin or CoQ_{10} treatment lowered the increase of heart weight induced by the high-fat diet (p<0.01). When the rats were treated with both Statin and 270 mg/kg CoQ_{10} (HSC270: 1.34 ± 0.03 g), the

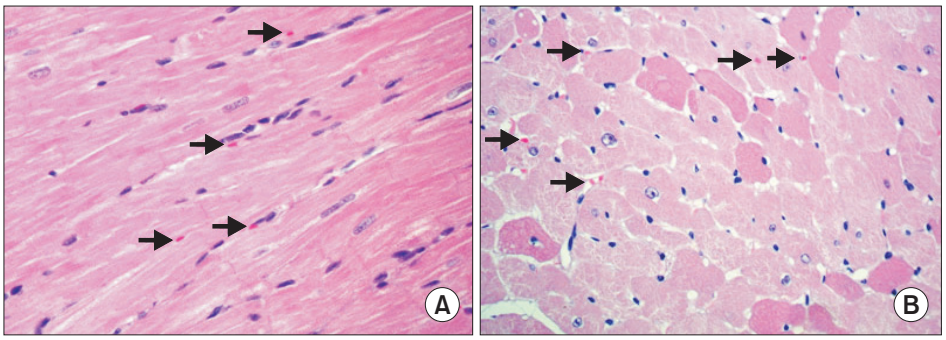

Fig. 2. Histological examination of heart tissues in the high-fat diet rats treated with Statin. Light microscopic finding/myocardium. In longitudinal (A) and transverse section (B), cardiac muscle fibers joined to each other by intercalated discs and having central nuclei and regular striations. 400×.

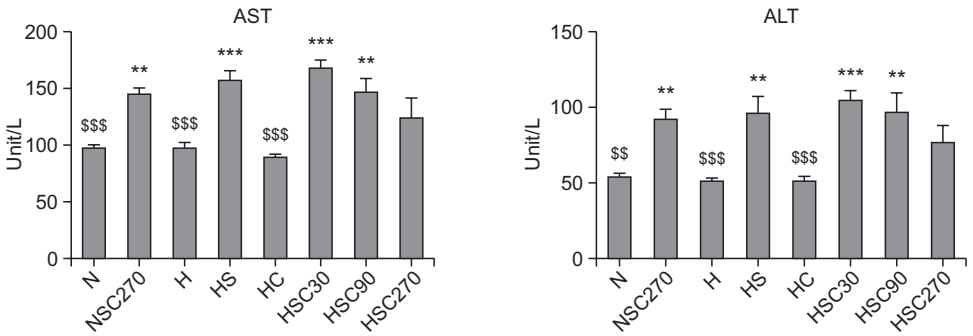

Fig. 3. Effect of CoQ_{10} supplementation on serum ALT and AST levels in Statin-treated obese rats. Data are mean ± S.E. (N=10). AST: aspartate aminotransferase, ALT: alanin aminotransferase. $^{**}p<0.01$, $^{***}p<0.001$ vs. normal diet group, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$ vs. high-fat diet with Statin group. N: normal diet group, NSC270: normal diet with Statin and CoQ_{10} (270 mg/kg) group, H: high-fat diet group, HS: high-fat diet with Statin group, HC: high-fat diet with CoQ_{10} (270 mg/kg) group, and HSC30, HSC90, HSC270: high-fat diet with Statin and CoQ_{10} at various doses (30, 90 or 270 mg/kg) group.

heart weights were decreased to as low as the normal diet group (N: 1.32 ± 0.02 g).

Heart histology

To test the effect of CoQ_{10} supplementation on myopathy, myolysis or rhabdomyolysis induced by Statin treatment, histological analysis of heart tissues from each group was performed. We failed to observe any significant pathological findings in the high-fat diet group (H) as well as the normal diet group (N). However, in Statin-treated high-fat diet group (HS), we observed mild non-inflammatory cellular changes including nuclei concentration and formation of vacuole in some cases (Fig. 2). Further microscopic examination is required to determine the effect of CoQ_{10}.

Serum AST and ALT levels

Along with the increase of creatine kinase levels, significantly elevated level of AST often suggests the existence of liver or heart disease such as hepatitis, congestive heart failure, bile duct problems or myopathy (Giboney, 2005). We observed a significant increase of AST level in Statin-treated group (HS, 157.30 ± 9.34 U/L) compared to the non-treated group (H, 98.10 ± 4.23 U/L, $p<0.001$), suggesting that Statin treatment induced muscle injuries in the high-fat diet group. However, CoQ_{10} (270 mg/kg) treatment significantly decreased serum AST level (Fig. 3). Serum ALT levels were also elevated by Statin treatment from 51.40 ± 2.04 U/L to 92.80 ± 10.86 U/L. Concurrent administration of CoQ_{10} with Statin (HSC) showed slightly increased ALT levels at 30 and 90 mg/kg dosages, however, we observed decrease of ALT levels in the 270 mg/kg CoQ_{10}-treated group (Fig. 3).

Effect of CoQ_{10} supplementation on CoQ_{10} and creatine kinase level in Statin-treated rats

It has been reported that CoQ_{10} depletion during statin therapy might be associated with subclonal cardiomyopathy, and that this situation is reversed upon CoQ_{10} treatment (Thompson *et al*., 2003; Littarru and Langsjoen, 2007; Marcoff and Thompson, 2007). Musculoskeletal side effects of statins include elevations in creatine kinase, myopathy, dermatomyositis and rhabdomyolysis. Other musculoskeletal side effects reported with HMG-CoA reductase inhibitors have included arthralgia, myalgia, tendon rupture, and dermatomyositis (Silva *et al*., 2006; Golomb and Evans, 2008). To test the effect of CoQ_{10} supplementation on the Statin-induced muscle injuries, we measured serum creatine kinase levels as well as ubiquinone levels (Table 2). Since the amount of CoQ_{10} in the high-fat diet group was already below the detection limit (H, <20 mg/ml), we were not able to observe further depletion of CoQ_{10} after Statin treatment (HS, <20 mg/ml). However, CoQ_{10} treatment dramatically increased the serum CoQ_{10} levels in the high-fat diet group (HC). Administration of CoQ_{10} with

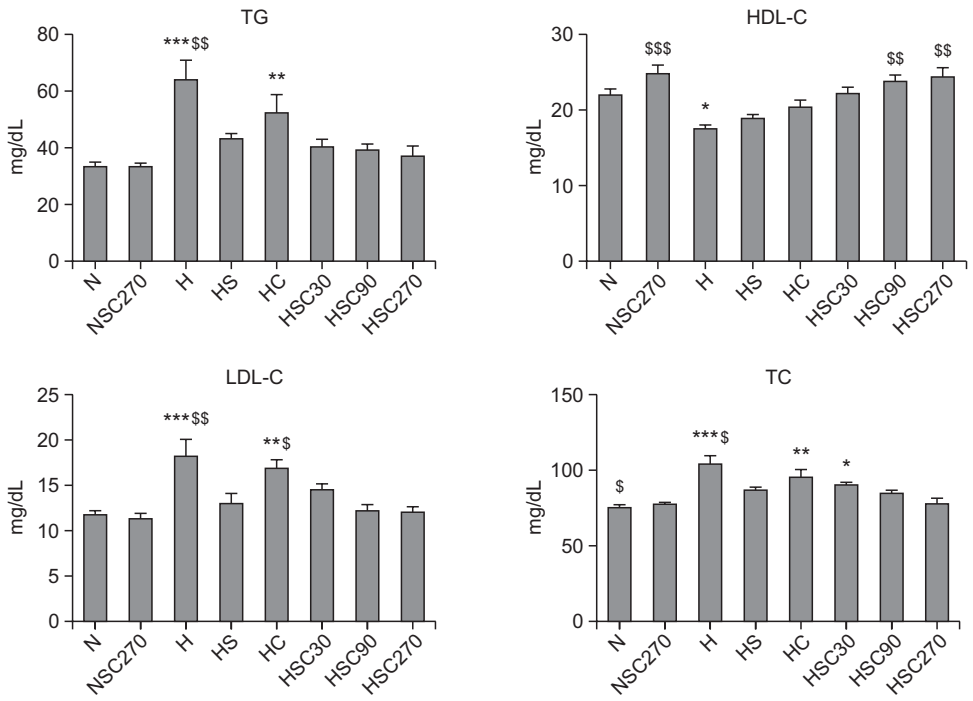

Fig. 4. Effect of CoQ_{10} supplementation on serum TG, HDL-C, LDL-C and TC levels in Statin-treated obese rats. Data are mean ± S.E. (N=10). TG: Triglyceride, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol, TC: Total cholesterol. *p<0.05, **p<0.01, ***p<0.001 vs. normal diet group, $^{\$}p$<0.05, $^{\$\$}p$<0.01, $^{\$\$\$}p$<0.001 vs. high-fat diet with Statin group. N: normal diet group, NSC270: normal diet with Statin and CoQ_{10} (270 mg/kg) group, H: high-fat diet group, HS: high-fat diet with Statin group, HC: high-fat diet with CoQ_{10} (270 mg/kg) group, and HSC30, HSC90, HSC270: high-fat diet with Statin and CoQ_{10} at various doses (30, 90 or 270 mg/kg) group.

Statin also significantly increased the levels of serum CoQ_{10} in a dose-dependent manner (HSC30, 90 and 270). Notably, administration of Statin in CoQ_{10}-treated obese rats dramatically attenuated CoQ_{10} level (HC vs HSC270), confirming the CoQ_{10}-depleting effect of Statin as previously reported.

A high-fat diet increased serum creatine kinase level from 400.10 ± 43.57 U/L (N) to 554.60 ± 31.51 U/L (H). Statin-treated rats showed further increase of creatine kinase level in serum (HS: 652.40 ± 36.40 U/L). Concurrent administration of CoQ_{10} with Statin (HSC) decreased serum creatine kinase levels in a dose-dependent manner, suggesting that Statin-induced muscle injury in the high-fat diet group was attenuated by the combined treatment of CoQ_{10} (Table 2).

Effect of CoQ_{10} on serum lipid profiles

To explore the effect of CoQ_{10} on lipid level in blood, we examined TC, TG, HDL-C and LDL-C, of each group after 6 week treatment (Fig. 4). Rats in the high-fat diet group (H) exhibited significantly higher TC, TG, LDL-C. As expected, the high-fat diet resulted in a decrease of HDL-C level in serum (N group vs H group, p<0.05). The increases of TC, TG and LDL-C levels by a high-fat diet were significantly reduced by Statin treatment. Concurrent treatment of CoQ_{10} with Statin (HSC 90 and HSC270) further decreased TC, TG and LDL-C levels in a dose-dependent manner and significantly increased HDL-C levels, suggesting that CoQ_{10} supplementation enhances the anti-hyperlipidemic effect of Statin.

DISCUSSION

Since their introduction to the market in 1987, statins have been used to treat various lipid disorders, such as hypercholesterolemia, hypertriglyceridemia, mixed dyslipidemia and homozygous familial hyperlipidemia (Anand, 2003; Niska and Han, 2009; Ray *et al*., 2010). Statins inhibit HMG-CoA reductase which converts HMG-CoA into mevalonic acid, a cholesterol precursor. When they bind to the active site of HMG-CoA reductase, statins induce conformational change of the enzyme, resulting in the blocking of its interaction with its natural substrate, HMG-CoA (Istvan and Deisenhofer, 2001).

Although statins are known to be well tolerated, long-term treatment of statins can produce a variety of adverse effects including the elevation of serum creatine kinase level, myopathy, myositis, myalgia, low incidence of rhabdomyolysis, and initiative or accelerated progression of cataracts and neoplasia (Buchholz *et al*., 2000). In contrast to their molecular mechanism in the treatment of hyperlipidemia through inhibition of HMG-CoA reductase, little is known about the mechanism by which statins produce muscle injuries. Recently, several possible mechanisms have been proposed: 1) reduction of the cholesterol content of skeletal muscle membrane, and 2) inhibition of farnesyl pyrophosphate, which is an intermediate for the production of ubiquinone (Coenzyme Q_{10}) (Thompson *et al*., 2003).

CoQ_{10} has been found to be a key component in mitochondrial function (Deichmann *et al*., 2010). Localized in the inner mitochondrial membrane, it facilitates electron transfer in

the generation of adenosine triphosphate (ATP) (Siciliano *et al*., 2007). It has also been shown that CoQ_{10} has anti-oxidant effect, preventing the oxidation of proteins, DNA, and lipids (Giboney, 2005).

Here, we demonstrated that CoQ_{10} supplementation can prevent the adverse effects of statins. 6 weeks of a high-fat diet induced obesity in SD rats. Serum lipid levels in the high-fat diet group exhibited increased creatine kinase (Table 2), as well as significantly higher TG, TC and LDL-C levels (Fig. 4). In a previous study, healthy volunteers treated with Simvastatin for 4 weeks have shown a 30% decrease of blood CoQ_{10} level (Laaksonen *et al*., 1995; Rundek *et al*., 2004). However, our study did not confirm a change of serum CoQ_{10} levels in Statin-treated rats due to low detection sensitivity (Table 2). Despite this limitation, our findings show that administration of Statin in CoQ_{10}-treated obese rats dramatically attenuated CoQ_{10} level, confirming the CoQ_{10}-depleting effect of Statin as previously reported, and raise the possibility that CoQ_{10} supplementation rescues the muscle injuries caused by Statin treatment. Co-administration of CoQ_{10} with Statin decreased body weight and liver weight in a dose-dependent manner in the high-fat (Supplemental Table 3) diet induced obese rats (Table 1). In addition, concurrent administration of Statin with CoQ_{10} (HSC) significantly decreased serum creatine kinase levels in a dose-dependent manner (Table 2). We also observed that CoQ_{10} treatment significantly decreased serum AST levels elevated by Statin (Fig. 3). The solubility of CoQ_{10} in water is extremely low, resulting in a poor oral bioavailability. It accounts for that the highest dose of CoQ_{10} improved biochemical parameters in our experiment.

It is noteworthy that treatment with CoQ_{10} also provided a significantly enhanced potency of Statin in serum lipid profiles. The increases of TC, TG and LDL-cholesterol by a high-fat diet were significantly reduced by Statin treatment as expected. Moreover, the combined treatment of Statin with CoQ_{10} (HSC90 and HSC270) further decreased TC, TG and LDL-C levels and significantly increased HDL-C levels (Fig. 4). These results are consistent with previous report that CoQ_{10} supplementation inhibits the peroxidation of LDL-C which may play a key role in its anti-atherogenic effects (Littarru and Tiano, 2007).

Therefore, it clearly appears that CoQ_{10} supplementation is beneficial in Statin treatment. Moreover, the possibility of a synergistic effect of Statins with CoQ_{10} supplementation should be further evaluated for clinical purposes.

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