RESEARCH ARTICLE

Supplementation with the reduced form of Coenzyme Q_{10} decelerates phenotypic characteristics of senescence and induces a peroxisome proliferator-activated receptor-α gene expression signature in SAMP1 mice

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Our present study reveals significant decelerating effects on senescence processes in middle-aged SAMP1 mice supplemented for 6 or 14 months with the reduced form ($Q_{10}H_2$, 500 mg/kg BW/day) of coenzyme Q_{10} (CoQ_{10}). To unravel molecular mechanisms of these CoQ_{10} effects, a genome-wide transcript profiling in liver, heart, brain and kidney of SAMP1 mice supplemented with the reduced ($Q_{10}H_2$) or oxidized form of CoQ_{10} (Q_{10}) was performed. Liver seems to be the main target tissue of CoQ_{10} intervention, followed by kidney, heart and brain. Stringent evaluation of the resulting data revealed that $Q_{10}H_2$ has a stronger impact on gene expression than Q_{10}, primarily due to differences in the bioavailability. Indeed, $Q_{10}H_2$ supplementation was more effective than Q_{10} to increase levels of CoQ_{10} in the liver of SAMP1 mice. To identify functional and regulatory connections of the "top 50" ($p<0.05$) $Q_{10}H_2$-sensitive transcripts in liver, text mining analysis was used. Hereby, we identified $Q_{10}H_2$-sensitive genes which are regulated by peroxisome proliferator-activated receptor-α and are primarily involved in cholesterol synthesis (*e.g.* HMGCS1, HMGCL and HMGCR), fat assimilation (FABP5), lipoprotein metabolism (PLTP) and inflammation (STAT-1). These data may explain, at least in part, the decelerating effects on degenerative processes observed in $Q_{10}H_2$-supplemented SAMP1 mice.

Keywords:
CoQ_{10} / Gene expression / Inflammation / Lipid metabolism / Peroxisome proliferator-activated receptor-α

1 Introduction

Coenzyme Q_{10} (CoQ_{10}) is an essential cofactor in the electron transport chain, serves as a potent antioxidant in lipid membranes and is a cofactor of uncoupling proteins. More recently, we identified CoQ_{10} as a compound with anti-inflammatory properties *in vitro* [1, 2]. These effects are thought to be mediated through gene expression and/or the radical scavenging activity of the reduced form of CoQ_{10} ($Q_{10}H_2$) [3, 4]. Moreover, $Q_{10}H_2$ also indicated stronger anti-inflammatory effects than the oxidized form (Q_{10}) *in vitro*. From these data, different effects of the oxidized and reduced form of CoQ_{10} on redox-dependent gene expression patterns were hypothesized. Very recently, we provide evidence *in vitro* and in mice that $Q_{10}H_2$ modulates the expression of the anti-inflammatory microRNA-146a [5].

The senescence-accelerated mice is a well-established model to study the aging process in higher organisms [6, 7]. SAMP

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Abbreviations: LXR, Liver X receptor; **PPAR-α,** peroxisome proliferator-activated receptor-α; **PPRE,** peroxisome proliferator response element; **RXR,** retinoid X receptor

strains grow normally but show early signs of aging including, *e.g.* reduced physical activity, loss of hair glossiness and shorter life span [8]. Analysis of aging dynamics, based on survival curves, senescence scores and growth rate, shows that the aging pattern in SAMP strains is characterized by accelerated senescence after normal development [8, 9]. Because SAMP1 mice show also a high oxidative stress status [10, 11], they are a suitable model to study putative effects of antioxidants such as CoQ_{10} on physiological and molecular readouts. Our present study shows significant reducing effects on accelerated senescence processes in SAMP1 mice supplemented with $Q_{10}H_2$. To get insight into the molecular mechanisms of these effects, we studied the influence of Q_{10} as well as $Q_{10}H_2$ on gene expression in liver, heart, brain and kidney of SAMP1 mice.

2 Materials and methods

2.1 Animals

SAMP1 mice were reared in the Division of Laboratory Animal Research, Research Center for Human and Environmental Sciences, Shinshu University, under specific pathogen-free conditions at $24 \pm 2^{\circ}C$ and a 12-h light–dark cycle. Water and food intake were available *ad libitum*. At the beginning of the long-term controlled study, 4-wk-old female SAMP1 mice were purchased from Japan SLC (Hamamatsu, Japan) and housed 3–6 *per* cage (20 cm wide, 30 cm long, 10 cm deep). Grouping of the animals remained unchanged throughout the study. Body weights, food intake and degree of senescence were calculated monthly while mice were inspected daily. Animals were sacrificed by cardiac puncture under anaesthesia with diethyl ether after 6 or 14 months of $Q_{10}H_2$ or Q_{10} supplementation. Organs (liver, heart, brain and kidney) were removed and stored at $-80^{\circ}C$ until RNA isolation. Study protocol and experimental procedures were approved by the ethics committee of Shinshu University.

2.2 Evaluation of degree of senescence

The degree of senescence was evaluated by a grading system [9]. Eleven categories of behavioral activity and gross appearances of the skin, eyes, and spine were considered to be associated with the aging process: each category was graded 0 to 4 according to the degree of change, and the grading score for each mouse was the sum of the grades of each category. Generally, the grading was done at a fixed time (from 2 pm to 4 pm) by an observer who was blinded to the treatment of the mice.

2.3 $Q_{10}H_2$ and Q_{10} supplementation and preparation of the diet

In the initial phase of the experiment, SAMP1 mice were randomly assigned to three groups: $Q_{10}H_2$ animals ($n = 22$), Q_{10} animals ($n = 11$) or control animals ($n = 20$).

The oxidized (Q_{10}) or reduced form ($Q_{10}H_2$) of CoQ_{10} was added to a standard laboratory mouse diet (powdered CE-2, CLEA Japan) using corn oil (1%, v/w) as a vehicle and to achieve a final concentration of Q_{10} or $Q_{10}H_2$ of 0.5%, respectively. The control diet was prepared using corn oil only. The mixture was incorporated in pellet-type chow by adding 30% v/v ethanol solution, pressure shaping and drying. Storage of the diet was conducted at $-20^{\circ}C$ for up to 4 wk before administration.

2.4 Determination of total CoQ_{10} and its redox state in liver samples of 14 M SAMP1 mice

Total levels and redox state of CoQ_{10} ($Q_{10}H_2$, Q_{10}) were determined in liver homogenates of 14 M intervention ($Q_{10}H_2$, Q_{10}) and control mice ($n = 3$ *per* each group). The method is based on HPLC analysis with electrochemical detection with minor modifications as described before [12]. In brief, 1.95 mL of 2-propanol was added to 50 mg liver sample and mixed with a Polytron homogenizer. Subsequently, the homogenate was diluted 15-fold with 2-propanol. After centrifugation ($9500 \times g$, 3 min, $4^{\circ}C$), 50 μL of the supernatant was injected into the HPLC system.

The mobile phase consisted of 0.05 M sodium perchlorate in methanol/hexane (88:12 v/v) at a flow rate of 1.0 mL/min. The oxidation potential of the ECD was 600 mV (*versus* Ag/AgCl). $Q_{10}H_2$ and Q_{10} levels were quantified by an external standard method based on peak area.

2.5 Isolation of mRNA from mice tissue for microarray analysis

Total RNA was extracted from mice tissues ($n = 3$ *per* each group) with the following kits (all from Qiagen, Japan) according to the manufacturer's instructions: RNeasy Kit (for liver and kidney), RNeasy Fibrous Tissue Mini Kit (for heart) and RNeasy Lipid Tissue Mini Kit (for brain).

2.6 Expression profiling

Microarray analysis was conducted on three samples for each group, respectively, by using GeneChip® Mouse Genome 430 2.0 Array (Affymetrix) containing 45 100 probe sets. The procedure was performed according to the manufacturer's instructions using Poly-A RNA Control Kit (Affymetrix) and One-Cycle cDNA Synthesis Kit (Affymetrix) for cDNA synthesis, Sample Cleanup Module (Affymetrix) for purification, and IVT Labeling Kit (Affymetrix) for synthesis of biotin-labeled cRNA. Fifteen micrograms of fragmented cRNA was hybridized to

a Mouse Genome 430 2.0 Array for 16 h at 45°C at 60 rpm. After hybridization, arrays were washed on GeneChip® Fluidics station 450 (Affymetrix) and stained with streptavidin–phycoerythrin. Thereafter, microarrays were scanned with a GeneChip® Scanner 3000 7G (Affymetrix). Expression data were normalized with Affymetrix GeneChip Operating Software 1.4 using mean value (global normalization). Only probe sets showing present calls for all three arrays at one experimental group (intervention or control) were considered for further analysis. The accession number for the complete datasets submitted to NCBI Gene Expression Omnibus is GSE15129 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = GSE15129).

2.7 Text-mining study-Genomatix bibliosphere

Genomatix Software 2008 (http://www.genomatix.de) was used to perform text-mining analysis. The probe set IDs of the $Q_{10}H_2$-regulated genes were uploaded to BibliospherePathwayEdition Software. This text-mining tool identifies putative functional connections based on co-citations of gene names and synonyms from NCBI Pubmed [13]. The co-citation filter "gene…function word…gene" (GFG level B3) was applied.

2.8 Statistics

Results were analyzed by an unpaired, two-sided Student's *t*-test using SPSS 11.5 for Windows and GraphPad Prism 4.0 software. *p*-Values less than or equal to 0.05 were considered statistically significant.

3 Results

3.1 Effects of $Q_{10}H_2$- and Q_{10}-supplementation on food intake and grading score of senescence in SAMP1 mice

Food intake of SAMP1 mice was calculated monthly and no difference in food consumption was apparent among the three groups (control, $Q_{10}H_2$ and Q_{10}). Based on mean food intake (3.4 g/day) and mean body weight (33 g), mice consumed 500 mg/kg BW/day $Q_{10}H_2$ or Q_{10}, respectively. No differences in body weight were found between $Q_{10}H_2$ and Q_{10} supplemented animals. Senescence grading scores increased from 16 or 20 wk of age in SAMP1 mice of all groups ($Q_{10}H_2$, Q_{10} and control). However, grading scores in the $Q_{10}H_2$ group were lower than in Q_{10}- and control-treated mice. There was a significant difference between control and $Q_{10}H_2$ mice from 16 to 60 wk of age ($p<0.05$) (Fig. 1). This result was also found in previous experiments [14].

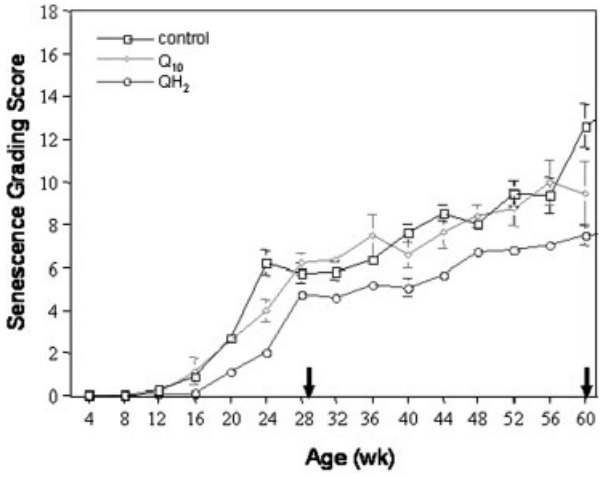

Figure 1. Age-related change on senescence grading scores in SAMP1 mice supplemented with $Q_{10}H_2$, Q_{10} or vehicle control. SAMP1 mice were supplemented with either $Q_{10}H_2$ or Q_{10} (~500 mg/kg BW/d), or a respective control diet for 14 months. Senescence grading scores increased from 16 or 20 wk of age in SAMP1 mice of all groups ($Q_{10}H_2$, Q_{10}, control). Grading scores in the $Q_{10}H_2$ group were lower than in Q_{10} and control mice. There was a significant difference ($p<0.05$) between control and $Q_{10}H_2$ mice from 16 to 60 wk of age ($p<0.05$). However, no significant differences were found for Q_{10}-treated animals when compared with controls.

3.2 Effects of $Q_{10}H_2$- and Q_{10}-supplementation on whole genome expression profiles in different tissues of SAMP1 mice

Microarray-based whole genome expression profiles were analyzed from liver, heart, brain and kidney of SAMP1 mice supplemented with $Q_{10}H_2$, Q_{10} or a control diet. From every experimental group, six mice *per* each group were sacrificed at 6 and 14 months (three mice at each time point, respectively) after supplementation, resulting in a total of 72 microarrays. Differentially expressed genes in tissues of SAMP1 mice treated with $Q_{10}H_2$ or Q_{10} were selected as follows. First, transcripts with at least three present calls in one group (intervention *versus* control) were chosen for further analysis. Second, transcripts showing at least a 1.5-fold increase or decrease in the $Q_{10}H_2$ or Q_{10} groups *versus* control animals at $p<0.05$ by Student's *t*-test, or alternatively, a 1.3-fold increase or decrease at $p<0.01$, were selected. Third, only those transcripts were referred to as $Q_{10}H_2$- and/or Q_{10}-sensitive having been significantly expressed in at least three different tissues, or alternatively, in two tissues at different time points or with a fold-change level of at least $\geq$I1.5I at $p<0.01$ (Tables 1 and 2). Based on these stringent criteria, 20 and 3 transcripts were identified as $Q_{10}H_2$- or Q_{10}-sensitive, respectively (Table 1). Most $Q_{10}H_2$-sensitive transcripts were differentially expressed in liver and kidney at 6 or 14 months after supplementation. Moreover, 17 transcripts were identified to be regulated by

Table 1. Expression profiles of $Q_{10}H_2$- and Q_{10}-sensitive transcripts in different tissues

	Fold change $Q_{10}H_2$								
	Liver		Heart		Brain		Kidney		
Gene ID	6M	14M	6M	14M	6M	14M	6M	14M	Gene name
228880						1.74**		2.15**	RIKEN CDNA 2010005I16 GENE
217232	2.10**						1.86**	1.45**	CELL DIVISION CYCLE 27 HOMOLOG (S. CEREVISIAE)
17979	3.64*						2.16*	1.59**	NUCLEAR RECEPTOR COACTIVATOR 3
108962	2.21*	1.76*					1.68*		RIKEN CDNA 4833441D16 GENE
20481	3.67**						2.36**		SLOAN-KETTERING VIRAL ONCOGENE HOMOLOG
72949	3.21**						1.82*	1.82**	CYCLIN T2
170942	2.60**			1.91*			1.96**	1.56*	ERYTHROID DIFFERENTIATION REGULATOR 1
100910	2.10**			2.18**			1.70*		RIKEN CDNA 2010209O12 GENE
66277		−1.70**	−1.59**						KRUPPEL-LIKE FACTOR 15
21413	3.19*						1.75*	1.77*	TRANSCRIPTION FACTOR 4
84092	2.28*	2.97*					1.56*		PUTATIVE DEUBIQUITINATING ENZYME
56490	3.53**							1.82**	ZINC FINGER AND BTB DOMAIN CONTAINING 20
NA	2.31*	1.69*					1.72**		NA
94112	2.93*						2.17**	1.69*	POSITIVE COFACTOR 2, MULTIPROTEIN COMPLEX, GLUTAMINE/Q-RICH-ASSOCIATED PROTEIN
170942	2.26**						2.02**	1.51*	ERYTHROID DIFFERENTIATION REGULATOR 1
NA	2.41**						2.60**		NA
319885				2.13*			1.82*	1.83*	ZINC FINGER, CCHC DOMAIN CONTAINING 7
67039	2.17*	2.60*					2.0*	1.66*	RIKEN CDNA 2600011C06 GENE
52680	1.88*					1.73*		1.53**	DNA SEGMENT, CHR 13, ERATO DOI 787, EXPRESSED
27981			1.95**				2.01**		DNA SEGMENT, CHR 4, WAYNE STATE UNIVERSITY 53, EXPRESSED
	Fold change Q_{10}								
	Liver		Heart		Brain		Kidney		
Gene ID	6M	14M	6M	14M	6M	14M	6M	14M	Gene name
15511		3.67*				2.17*		4.14**	HEAT SHOCK PROTEIN 1B
94089		2.17**					3.63**		TRIPARTITE MOTIF PROTEIN 7
76044	1.69*				1.69*		1.66*		LEUCINE ZIPPER PROTEIN 5

*$p \leq 0.05$, **$p \leq 0.01$

$Q_{10}H_2$ as well as Q_{10} treatment (Tables 2 and 3). These transcripts were designated as ''CoQ_{10}-sensitive.'' As shown in Tables 2 and 3, 14 CoQ_{10}-sensitive transcripts were differentially expressed in the kidney at 6 months but not at 14 months after supplementation with Q_{10} and $Q_{10}H_2$. In the liver, 16 CoQ_{10}-sensitive transcripts were affected by Q_{10} at 14 months after supplementation. In contrast, $Q_{10}H_2$ affected most of these genes at 6 months after supplementation. Taken together, our data in SAMP1 mice suggested that liver and kidney are the main target tissues of $Q_{10}H_2$ or Q_{10} intervention regarding gene expression. Furthermore, $Q_{10}H_2$ may have stronger impact on gene expression. In accordance to this hypothesis, $Q_{10}H_2$-sensitive transcripts (liver, 14 M supplemented) which suggest stronger regulatory effects (''Top 10,'' $p < 0.05$) are characterized by higher expression fold-change values in comparison to Q_{10}-sensitive transcripts (Tables 4 and 5).

3.3 Detailed analysis of gene expression data obtained from liver samples of $Q_{10}H_2$-supplemented SAMP1 mice

With regard to the effects of $Q_{10}H_2$ and Q_{10} on gene expression in different tissues of SAMP1 mice, liver was shown to be strongly affected. Regarding the senescence deceleration process in SAMP1 mice, $Q_{10}H_2$ was more effective than Q_{10} (Fig. 1). This was also shown by a previous study [14]. To study long-term effects of $Q_{10}H_2$-supplementation on gene expression in more detail, liver samples of $Q_{10}H_2$-supplemented animals (14 M) were used. Initially, up and downregulated $Q_{10}H_2$-sensitive transcripts displaying the highest fold-change values (''Top 50,'' $p < 0.05$) were selected. To unravel the functional connections of these genes we performed a text mining approach using the Genomatix BibliospherePathwayEdition Software

Table 2. Expression profiles of transcripts both sensitive for $Q_{10}H_2$ and Q_{10} in different tissues: fold change $Q_{10}H_2$

	Fold change $Q_{10}H_2$								
	Liver		Heart		Brain		Kidney		
Gene ID	6M	14M	6M	14M	6M	14M	6M	14M	Gene name
63830#	3.34*#	3.87*					2.31*#		KCNQ1 OVERLAPPING TRANSCRIPT 1
552902				1.67*			1.92**		HYPOTHETICAL LOC552902
68186	2.87*						2.03*		RIKEN CDNA 4632427E13 GENE
233489							2.43*		PHOSPHATIDYLINOSITOL BINDING CLATHRIN ASSEMBLY PROTEIN
78265	2.92**						2.57**		RIKEN CDNA 4632418H02 GENE
27981			2.12*				2.61**		DNA SEGMENT, CHR 4, WAYNE STATE UNIVERSITY 53, EXPRESSED
68371	3.41**						1.91*		RIKEN CDNA 0610038K03 GENE
96982	3.48*							1.85**	EXPRESSED SEQUENCE C79248
76719	2.51**						1.92**		RIKEN CDNA 1700081L11 GENE
319263	2.61**			1.59*		1.52*		1.66*	PROTEIN-L-ISOASPARTATE (D-ASPARTATE) O-METHYLTRANSFERASE DOMAIN CONTAINING 1
20239		2.00**					1.60*		ATAXIN 2
208618	4.26*						1.87*		CDNA SEQUENCE BC026657
108829	2.40*							1.66*	JUMONJI DOMAIN CONTAINING 1C
622943						1.67*	2.24**		DNA SEGMENT, CHR 5, ERATO DOI 579, EXPRESSED
320861	4.15*						1.60*		RIKEN CDNA C130047D21 GENE
13196		2.21*					2.01*		DEVELOPMENT AND DIFFERENTIATION ENHANCING
72739	2.41**						2.43**		ZINC FINGER PROTEIN 306

*$p \leq 0.05$, **$p \leq 0.01$, #presented by ≥ 2 probe set IDs.

Table 3. Expression profiles of transcripts both sensitive for $Q_{10}H_2$ and Q_{10} in different tissues: fold change Q_{10}

	Fold change Q_{10}								
	Liver		Heart		Brain		Kidney		
Gene ID	6M	14M	6M	14M	6M	14M	6M	14M	Gene name
63830#		1.64*					3.07*		KCNQ1 OVERLAPPING TRANSCRIPT 1
552902		1.89*					1.71**		HYPOTHETICAL LOC552902
68186		1.51*					2.04*		RIKEN CDNA 4632427E13 GENE
233489		2.59*					2.79*		PHOSPHATIDYLINOSITOL BINDING CLATHRIN ASSEMBLY PROTEIN
78265		1.86**					3.34*		RIKEN CDNA 4632418H02 GENE
27981			2.31**				2.23*		DNA SEGMENT, CHR 4, WAYNE STATE UNIVERSITY 53, EXPRESSED
68371		1.90*					1.54*		RIKEN CDNA 0610038K03 GENE
96982		1.73*					2.18**		EXPRESSED SEQUENCE C79248
76719		1.51*					1.95**		RIKEN CDNA 1700081L11 GENE
319263		2.03*					1.53*		PROTEIN-L-ISOASPARTATE (D-ASPARTATE) O-METHYLTRANSFERASE DOMAIN CONTAINING 1
20239		2.53**					1.92**		ATAXIN 2
208618		2.46*					1.71*		CDNA SEQUENCE BC026657
108829		1.77*					2.08*		JUMONJI DOMAIN CONTAINING 1C
622943		1.94*					2.51**		DNA SEGMENT, CHR 5, ERATO DOI 579, EXPRESSED
320861		2.25*					1.54*		RIKEN CDNA C130047D21 GENE
13196		1.66**					1.84*		DEVELOPMENT AND DIFFERENTIATION ENHANCING
72739		1.77*					2.57**		ZINC FINGER PROTEIN 306

*$p \leq 0.05$, **$p \leq 0.01$, #presented by ≥ 2 probe set IDs.

Table 4. ''Top 10'' of up- and down-regulated $Q_{10}H_2$-regulated transcripts in the liver of SAMP1 mice (14M)

Gene ID	FC	Gene symbol	Gene name
Upregulated			
21822	21.24*	TGTP	T-CELL SPECIFIC GTPASE
27007	14.18*	KLRK1	KILLER CELL LECTIN-LIKE RECEPTOR SUBFAMILY K, MEMBER 1
18439	12.52*	P2RX7	PURINERGIC RECEPTOR P2X, LIGAND-GATED ION CHANNEL, 7
19363	12.07*	RAD51L1	RAD51-LIKE 1 (S. CEREVISIAE)
328563	10.68**	APOL11B	RIKEN CDNA A330102K04 GENE
22368	9.68*	TRPV2	TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL, SUBFAMILY V, MEMBER 2
328563	9.55*	APOL11B	RIKEN CDNA A330102K04 GENE
20846	9.21*	STAT1	SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 1
11801	9.11*	CD5L	CD5 ANTIGEN-LIKE
14131	8.87*	FCGR3	FC RECEPTOR, IGG, LOW AFFINITY III
Downregulated			
17840#	−6.12**	MUP1	MAJOR URINARY PROTEIN 1
56631	−4.60**	TRIM17	TRIPARTITE MOTIF PROTEIN 17
18113	−4.16*	NNMT	NICOTINAMIDE N-METHYLTRANSFERASE
23985	−4.16**	SLC26A4	SOLUTE CARRIER FAMILY 26, MEMBER 4
13089	−3.47**	CYP2B13	CYTOCHROME P450, FAMILY 2, SUBFAMILY B, POLYPEPTIDE 13
78894	−3.40*	AACS	ACETOACETYL-COA SYNTHETASE
76574	−3.38*	MFSD2	MAJOR FACILITATOR SUPERFAMILY DOMAIN CONTAINING 2
17844	−3.30*	MUP5	MAJOR URINARY PROTEIN 5
53901	−3.27**	RCAN2	DOWN SYNDROME CRITICAL REGION GENE 1-LIKE 1
13897	−3.26*	ES22	ESTERASE 22

*$p \leq 0.05$, **$p \leq 0.01$, #presented by ≥ 2 probe set IDs.

Table 5. ''Top 10'' of Q_{10} up and downregulated transcripts in the liver of SAMP1 mice (14M)

Gene ID	FC	Gene symbol	Gene name
Upregulated			
70945	7.15**	MMRN1	MULTIMERIN 1
NA	5.37**	NA	NA
207921	4.48*	A830093I24RIK	RIKEN CDNA A830093I24 GENE
381280	4.19*	6430706D22RIK	RIKEN CDNA 6430706D22 GENE
217166	4.00*	NR1D1	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP D, MEMBER 1
15511	3.90*	HSPA1B	HEAT SHOCK PROTEIN 1B
52822	3.86**	RUFY3	RUN AND FYVE DOMAIN CONTAINING 3
71972	3.85*	DNMBP	RIKEN CDNA 2410003L07 GENE
67039	3.84*	RBM25	RIKEN CDNA 2600011C06 GENE
11430	3.78**	ACOX1	ACYL-COENZYME A OXIDASE 1, PALMITOYL
Downregulated			
12592	−5.13**	CDX4	CAUDAL TYPE HOMEO BOX 4
16625	−3.90*	SERPINA3C	SERINE (OR CYSTEINE) PEPTIDASE INHIBITOR, CLADE A, MEMBER 3C
14803	−3.09*	GRID1	GLUTAMATE RECEPTOR, IONOTROPIC, DELTA 1
233987	−2.38*	BC003267	CDNA SEQUENCE BC003267
22648	−2.33*	ZFP11	ZINC FINGER PROTEIN 11
75458	−2.30*	CMTM2A	RIKEN CDNA 1700001K04 GENE
66658	−2.21**	CCDC51	COILED-COIL DOMAIN CONTAINING 51
216805	−2.18*	FLCN	FOLLICULIN
81011	−2.15*	V1RD14	VOMERONASAL 1 RECEPTOR, D14
75424	−2.04*	ZFP820	HYPOTHETICAL GENE MGC29393

*$p \leq 0.05$, **$p \leq 0.01$.

(GFG level B3). Co-cited transcripts having been represented by multiple probe sets for one gene and/or were shown by high intensity levels were selected. Based on these criteria, we identified 11 $Q_{10}H_2$-sensitive transcripts which seem to be primarily involved in cholesterol and lipid metabolism as well as in inflammatory processes and cell differentiation (Table 6). Moreover, a part of the identified $Q_{10}H_2$-sensitive genes is functionally connected by the

Table 6. Identification of $Q_{10}H_2$-sensitive genes and their functional connections

Gene ID	FC $Q_{10}H_2$	FC Q_{10}	Gene symbol	Gene name
Fatty acid and cholesterol synthesis				
208715#	−1.68*	n.s.	HmgCs1	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1
15356	−1.40*	n.s.	HmgCl	3-Hydroxy-3-methylglutaryl-coenzyme A lyase
15357	−2.44*	n.s.	HmgCr	3-Hydroxy-3-methylglutaryl-coenzyme A reductase
78894	−3.40*	n.s.	Aacs	Acetoacetyl-CoA Synthetase
20787	−1.97*	−1.52*	Srebf1	Sterol regulatory element-binding factor-1
Lipid mobilization				
16592#	3.63**	n.s.	Fabp5	Fatty acid binding protein 5, epidermal
Lipoprotein metabolism				
18830#	8.30*	n.s.	Pltp	Phospholipid transfer protein
Inflammation				
20846	9.21*	n.s.	Stat1	Signal transducer and activator of transcription 1
Cell differentiation and activation				
75104#	−3.17*	n.s.	Mmd2	Monocyte to macrophage differentiation-associated 2
17476	7.20*	n.s.	Mpeg1	Macrophage-expressed gene 1
100702	5.74**	n.s.	Mpa2l	Macrophage activation-2 like

*$p \leq 0.05$, **$p \leq 0.01$, #presented by ≥ 2 probe set IDs.

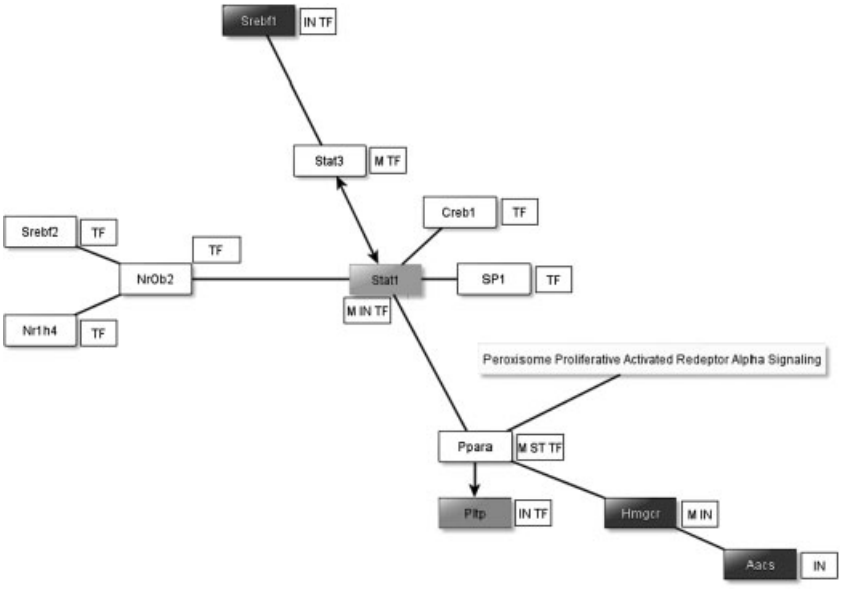

Figure 2. Bibliosphere network of $Q_{10}H_2$-sensitive genes regulated in the liver of SAMP1 mice. Based on co-citations with transcription factors and functional co-citations with other genes in the network (GFG level B3), 5 $Q_{10}H_2$-inducible genes were connected with each other by BibliospherePathwayEdition Software. According to this, the uploaded genes seem to play a key role in PPAR-α signaling. IN, input gene; TF, transcription factor; M, gene product is part of a metabolic pathway; ST, gene product is part of a Genomatix signal transduction pathway.

peroxisome proliferator-activated receptor-α (PPAR-α) signaling pathway (Fig. 2).

3.4 Accumulation of CoQ_{10} in liver samples of $Q_{10}H_2$- and Q_{10}-supplemented SAMP1 mice

Next, we tested whether CoQ_{10} accumulates in the liver of SAMP1 mice supplemented for 14 months with $Q_{10}H_2$ or Q_{10}. Total CoQ_{10} as well as its redox state was determined in liver homogenates of SAMP1 mice which were used for microarray experiments ($n = 3$ *per* each group). In comparison to control animals, total CoQ_{10} levels increased about 54.92-fold ($p = 0.0027$) and 30.36-fold ($p = 0.0006$) in the liver of $Q_{10}H_2$- and Q_{10}-supplemented mice, respectively (Fig. 3A). 77.53% ($Q_{10}H_2$-supplemented) and 80.72% (Q_{10}-supplemented) of CoQ_{10} was present in its reduced form. Because CoQ_9 is the predominant CoQ form in rodents [15], CoQ_9 levels were additionally determined in liver samples. As shown in Fig. 3D, the CoQ_9 concentration did not significantly change between treatment and control groups. In conclusion, $Q_{10}H_2$

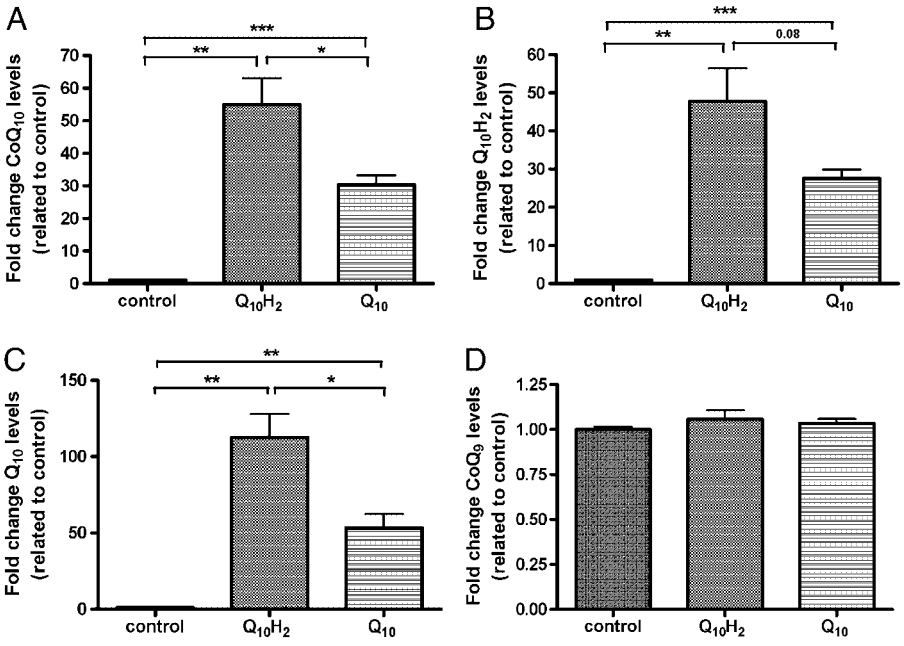

Figure 3. Effect of Q_{10} and $Q_{10}H_2$ supplementation on levels of total CoQ_{10} (A), redox status (B, C) and CoQ_9 (D) in liver tissues of SAMP1 mice. SAMP1-mice were supplemented with either $Q_{10}H_2$ or Q_{10} (500 mg/kg BW/d), or a respective control diet for 14 months. Thereafter, liver samples were collected, homogenized and used for HPLC analysis. Total CoQ_{10} levels increased about 54.92-fold ($p = 0.0027$) and 30.36-fold ($p = 0.0006$) in liver tissues of $Q_{10}H_2$- and Q_{10}-supplemented mice (A). Supplementation with $Q_{10}H_2$ and Q_{10} increased $Q_{10}H_2$ levels significantly about 47.86-fold ($p = 0.0054$) and 27.54-fold ($p = 0.0003$), respectively (B). $Q_{10}H_2$ supplementation induces also the strongest increase of Q_{10} levels in liver when related to controls (112.10-fold, $p = 0.0019$) (C). The CoQ_9 level did not significantly change between treatment and control groups (D). All data are means ± SEM of three animals *per* each group ($Q_{10}H_2$, Q_{10}, control), respectively.

supplementation was more effective than Q_{10} to increase the absolute levels of $Q_{10}H_2$ and Q_{10} in the liver of SAMP1 mice. Thereby, $Q_{10}H_2$ was the predominant form of CoQ_{10} in liver tissues.

4 Discussion

Data from previous [14] and present experiments reveal significant effects on decelerated senescence processes in SAMP1 mice supplemented with $Q_{10}H_2$ (Fig. 1). Additionally, distinct differences in gene expression profiles of $Q_{10}H_2$- and Q_{10}-supplemented SAMP1 mice in liver, heart, brain and kidney were identified. Because the oxidized form of CoQ_{10} can be reduced to $Q_{10}H_2$ by the plasma membrane redox system [16, 17], different effects of $Q_{10}H_2$ and Q_{10} on aging processes and gene expression are not obvious. However, the conversion of Q_{10} to $Q_{10}H_2$ is accompanied by the generation of reactive oxygen species [18],] which affects cellular redox-dependent gene regulation cascades [19]. A study in platelets also indicated a less effectiveness of the oxidized form of CoQ_{10} on oxidative stress parameters, despite the presence of quinone reductase activities [20]. Indeed, the activity of the plasma membrane redox system is modulated by different conditions including oxidative stress and aging [21, 22]. However, studies in perfused rat liver and isolated rat hepatocytes clearly indicated an antioxidant effect of exogenous $Q_{10}H_2$ [23, 24]. Some studies also reported about age-related decreases of CoQ_{10} levels in organs of both rats and humans [25]. In general, aging is considered as a process that seems to require an increase of antioxidant defenses to cope enhanced oxidative stress conditions [26, 27]. Additionally, differences in the tissue-dependent bioavailability of $Q_{10}H_2$ and Q_{10} may also have an impact on gene expression. Indeed, we found that $Q_{10}H_2$ supplementation was more effective than Q_{10} to increase levels of CoQ_{10} in the liver of SAMP1 mice. This might be in agreement with our previous results in monocytic cell lines, where incubation with raising doses of $Q_{10}H_2$ also induced a dose-dependent increase of the intracellular $Q_{10}H_2/Q_{10}$ ratio [5] when compared with Q_{10} incubation [1]. This $Q_{10}H_2$-specific effect additionally reveals the effectiveness of the exerted $Q_{10}H_2$ storage conditions for *in vitro* and *in vivo* studies. With regard to our present study, liver seems to be the main target tissue of CoQ_{10} intervention regarding gene expression, followed by kidney, heart and brain. This might be due to the fact that CoQ_{10} is mainly incorporated in LDL [28], which are taken up by the liver. In this context, it was also shown that CoQ_{10} is capable to prevent LDL particles from oxidation *in vitro* and *in vivo* [29–32]. This effect is considered to be mediated through the radical scavenging activity of the reduced form of CoQ_{10}, at least in part by the regeneration of tocopheryl radicals [31, 33–35]. In this context our preliminary data (unpublished results) from

another study in mice (C57BL6J) revealed a significant reduction (about $45 \pm 11.9\%$, $p = 0.0140$) of the pro-inflammatory chemokine MCP-1 in $Q_{10}H_2$ supplemented animals.

The observed consistence of CoQ_9 levels in tissue samples of CoQ_{10} supplemented mice were already described before [33].

With regard to gene expression data, a detailed analysis of whole genome expression profiles was performed for liver samples of mice supplemented with $Q_{10}H_2$ for 14 months. The results of the applied text-mining tool indicate an involvement of $Q_{10}H_2$-sensitive genes in the PPAR-α signaling pathway (Fig. 2). PPARs belong to the group of nuclear receptors and are negative regulators of numerous genes involved in lipid metabolism and cholesterol synthesis [36–40]. Moreover, it was shown that PPAR-α is predominantly expressed in tissues with high lipid catabolic activity [37]. In this context we identified a number of genes (Table 6) strongly downregulated by $Q_{10}H_2$-supplementation in the liver of SAMP1 mice. These genes were primarily involved in fatty acid and cholesterol synthesis (*e.g.* HMGCS1, HMGCL and HMGCR), lipid metabolism (FABP5) as well as lipoprotein metabolism (PLTP). Moreover, a regulatory role of PPAR-α in lipid metabolism and inflammatory processes is indicated in the literature [41–45]. The activation of PPAR-α occurs through, *e.g.* fatty acids and fibrates, a known class of hypolipidemic drugs. PPAR-α forms a heterodimer with retinoid X receptor (RXR) enhancing its binding to DNA sequence elements termed peroxisome proliferator response elements (PPRE) [46]. Liver X receptor (LXR) was found to inhibit the binding of the PPAR-α-9-cis retinoic acid receptor (PPAR-α/RXR) complex to PPRE [37]. Thus, ligand binding of LXR inhibits PPAR-α signaling and the activation of its downstream-target genes. Because LXR is activated by oxidized sterol and cholesterol metabolites [47, 48], antioxidant compounds might effectively inhibit activated LXR/RXR heterodimerization. Based on this data, we propose three putative functions for $Q_{10}H_2$ in PPAR-α-mediated signaling processes (Fig. 4): (i) as an antioxidant, leading to decreased levels of the LXR agonist oxidized LDL; (ii) as an antagonist of LXR, leading to PPAR-α/RXR heterodimers and PPRE activation; and (iii) as an agonist of PPAR-α, leading to PPAR-α/RXR heterodimers and PPRE activation. Moreover, the protein product of the Sterol regulatory element binding transcription factor-1 gene (SREBF-1, SREBP-1), described as a primary target gene of the RXR/LXR heterodimer [48, 49], was downregulated in liver samples of $Q_{10}H_2$-supplemented animals (Table 6). This might be a first indication of a $Q_{10}H_2$-mediated effect on PPAR-α signaling that leads to a reduced activation of the SREBF-1 promoter, a transcription factor that is known to activate fatty acid synthesis by increasing transcription of lipogenic genes [50–52]. Data of a recent study also demonstrate effects of CoQ_{10} treatment on lipid metabolism in obese ob/ob mice [53]. These effects were supposed to be mediated by PPAR-mediated activity. Moreover, a connection between PPAR-α signaling, inflammatory processes and neurodegenerative diseases in aging rats has been previously described [54]. These metabolic effects may explain, at least in part, the observed diminished effects on senescence characteristics in $Q_{10}H_2$-supplemented SAMP1 mice. Noteworthy is that the identified regulation of PPAR-α related genes observed in $Q_{10}H_2$-supplemented SAMP1 mice was not found in liver samples of Q_{10}-supplemented mice,

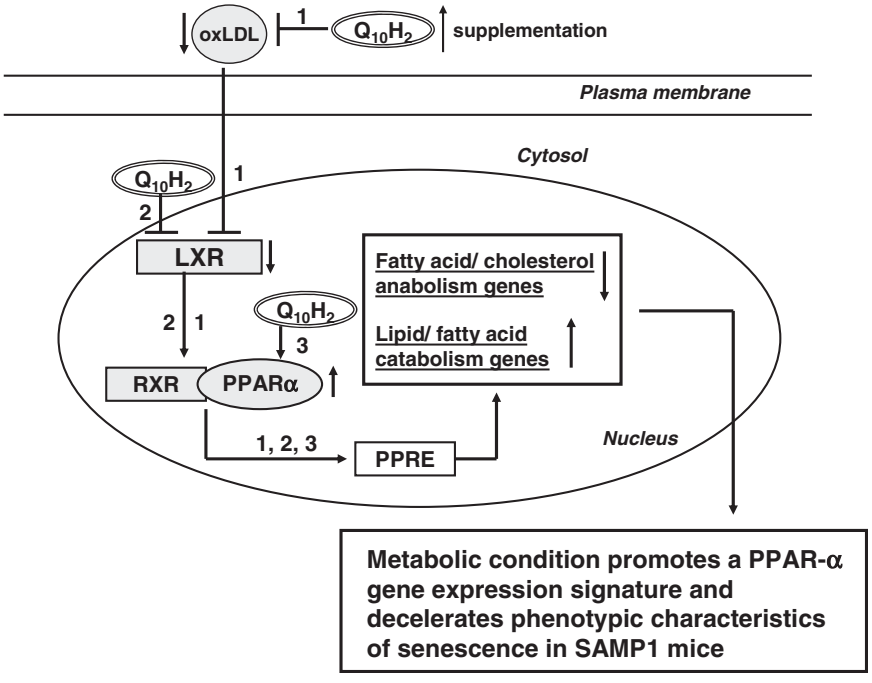

Figure 4. Putative mechanisms of $Q_{10}H_2$ action on PPAR-α signaling in liver tissues of SAMP1 mice. Three putative functions are proposed for $Q_{10}H_2$ in PPAR-α signaling: 1. As an antioxidant, leading to decreased levels of the LXR agonist oxidized LDL; 2. As an antagonist of LXR, leading to PPAR-α/RXR heterodimers and PPRE activation; and 3. As an agonist of PPAR-α, leading to PPAR-α/RXR heterodimers and PPRE activation.

indicating a $Q_{10}H_2$-specific effect. Hence, between $Q_{10}H_2$ and control animals, no differences in expression levels of PPAR-α have been identified (fold-change: 1.06, $p = 0.827$). This might be a further hint for a $Q_{10}H_2$-modulatory effect on PPAR-α at the protein level, the most important mechanism of transcription factor regulation.

Even if the exact mechanism of $Q_{10}H_2$-PPAR-α interaction cascades is not definitely clear, these results support our conclusions regarding a regulatory role of $Q_{10}H_2$ in PPAR-α signaling processes.

The authors have declared no conflict of interest.

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