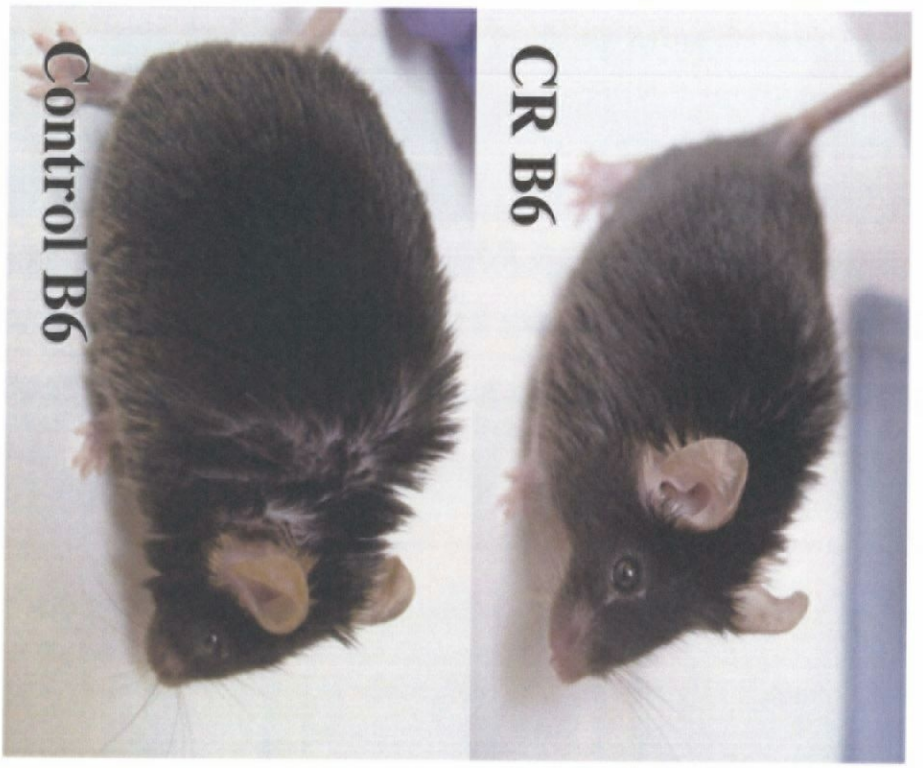
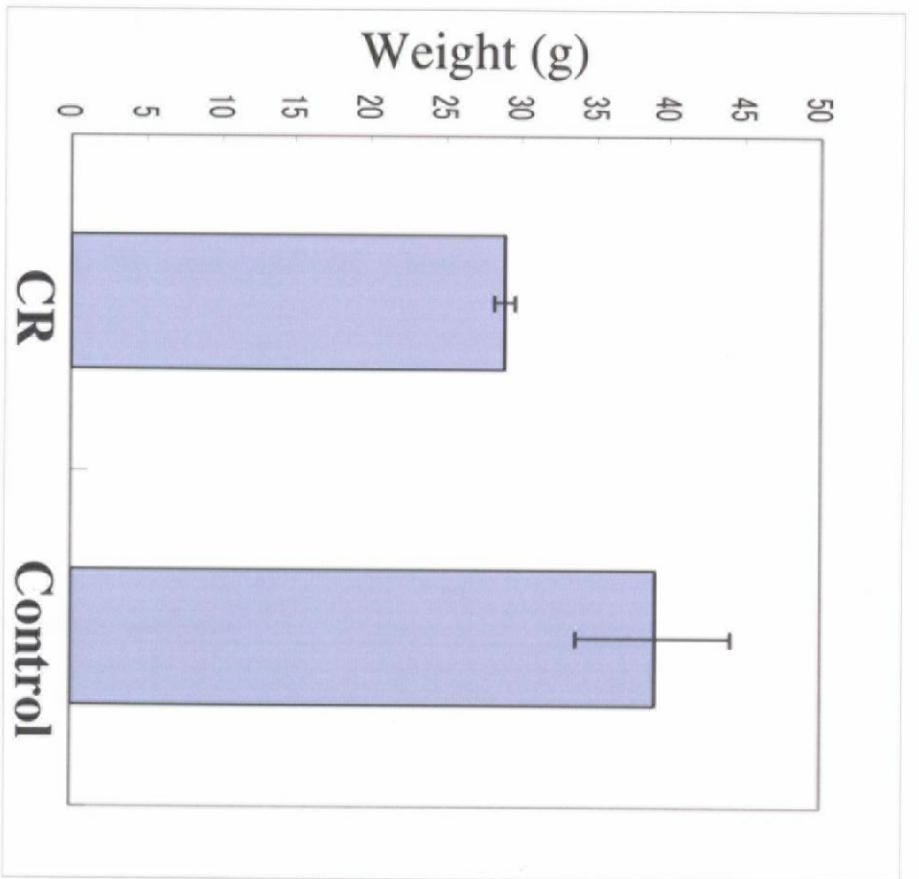
Chapter 5: Caloric restriction retards the progression of AHL by induction of *Sirt1*

Introduction

AHL is an important problem in society. In the US, it is estimated that approximately 30-35 percent of people between 65 and 75 years of age, and 40-50 percent of people over 75 years of age, have a hearing loss (NIDCD, 2004; Seidman, 2002). Nonetheless, no preventive or therapeutic interventions have been established for AHL (NIDCD, 2004; Seidman, 2002; Seidman, 2000; Seidman *et al.*, 2000; Seidman *et al.*, 2002). CR retards the aging process in laboratory animals as characterized by a delayed occurrence or complete prevention of a broad spectrum of age-associated pathophysiological changes, such as reduced weight (Figure 5-1), and a 30-50% increase in maximum lifespan (Lee *et al.*, 2002; Lee *et al.*, 1999; Lee *et al.*, 1999; Prolla, 2002; Kayo *et al.*, 2001). The maximum lifespan of fish, rotifers, spiders, and other non-mammals is also extended by CR (Lee *et al.*, 2002; Prolla, 2002). CR is the only intervention known to retard aging in mammals, and results in delayed onset of age-associated pathological alterations (Lee *et al.*, 1999a, 1999b, 2002; Prolla, 2002). Fowler et al. examined effects of caloric restriction on the auditory function of rohesus monkeys and showed that CR resulted in delayed onset of hearing loss (Fowler *et al.*, 2000). To understand how AHL can be retarded, I examined

Figure 5-1. CR C57BL/6 mouse showing reduced weight. The 15-month-old calorie-restricted C57BL/6 (B6) mouse appears leaner and smaller than the 15-month-old control B6 mouse.


CR B6
Control B6


Weight (g)
0
5
10
15
20
25
30
35
40
45
50
CR
Control

effects of CR on the progression of AHL by conducting ABR analysis, histological analysis, and gene expression profiling using B6 mice.

ABR analysis

To assess hearing impairment, ABR thresholds were measured at 4 kHz, 8 kHz, and 16 kHz. The mean of ABR thresholds for the control B6 mice (15 months of age) were 59.2 dB SPL at 4 kHz, 41.7 dB SPL at 8 kHz, and 35.0 dB SPL at 16 kHz, exhibiting moderate hearing loss (Figure 5-2). The mean of ABR thresholds for the CR B6 mice (15 months of age) were 20.8 dB SPL at 4 kHz, 22.5 dB SPL at 8 kHz, and 14.2 dB SPL at 16 kHz, indicating preservation of good hearing (Figure 5-2). These results suggest that CR retards the progression of AHL.

Histology

Histological analysis revealed that CR B6 mice exhibited virtually no degeneration of the organ of Corti, whereas control B6 mice exhibited degeneration of tissue integrity of the organ of Corti, and marked loss of spiral ganglion cells in the cochlea (Figure 5-3). These results support the ABR findings, and indicate that CR retards the progression of AHL.

Gene expression analysis

To examine transcriptional changes induced by CR, we used oligonucleotide arrays representing 45,037 genes and ESTs. A comparison of cochlea from the control B6 mice and the CR B6 mice revealed that CR is

Figure 5-2. Comparison of ABR thresholds. ABR thresholds were measured at 4 kHz, 8 kHz, and 16 kHz. The 15-month-old control C57BL/6 (B6) mice (n =6) showed moderate hearing loss, whereas the 15-month-old CR C57BL/6 (B6) mice (n = 6) retained good hearing.

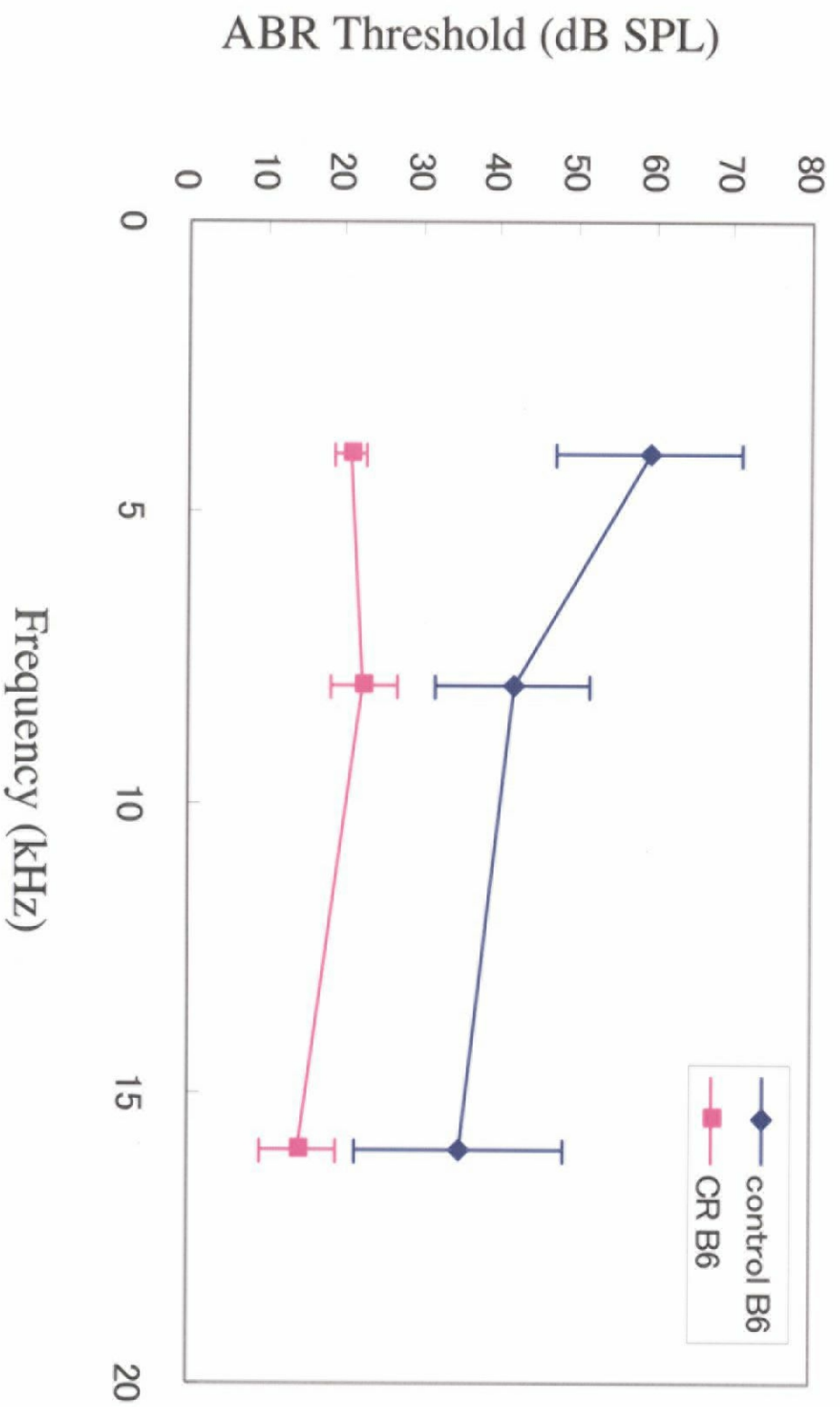
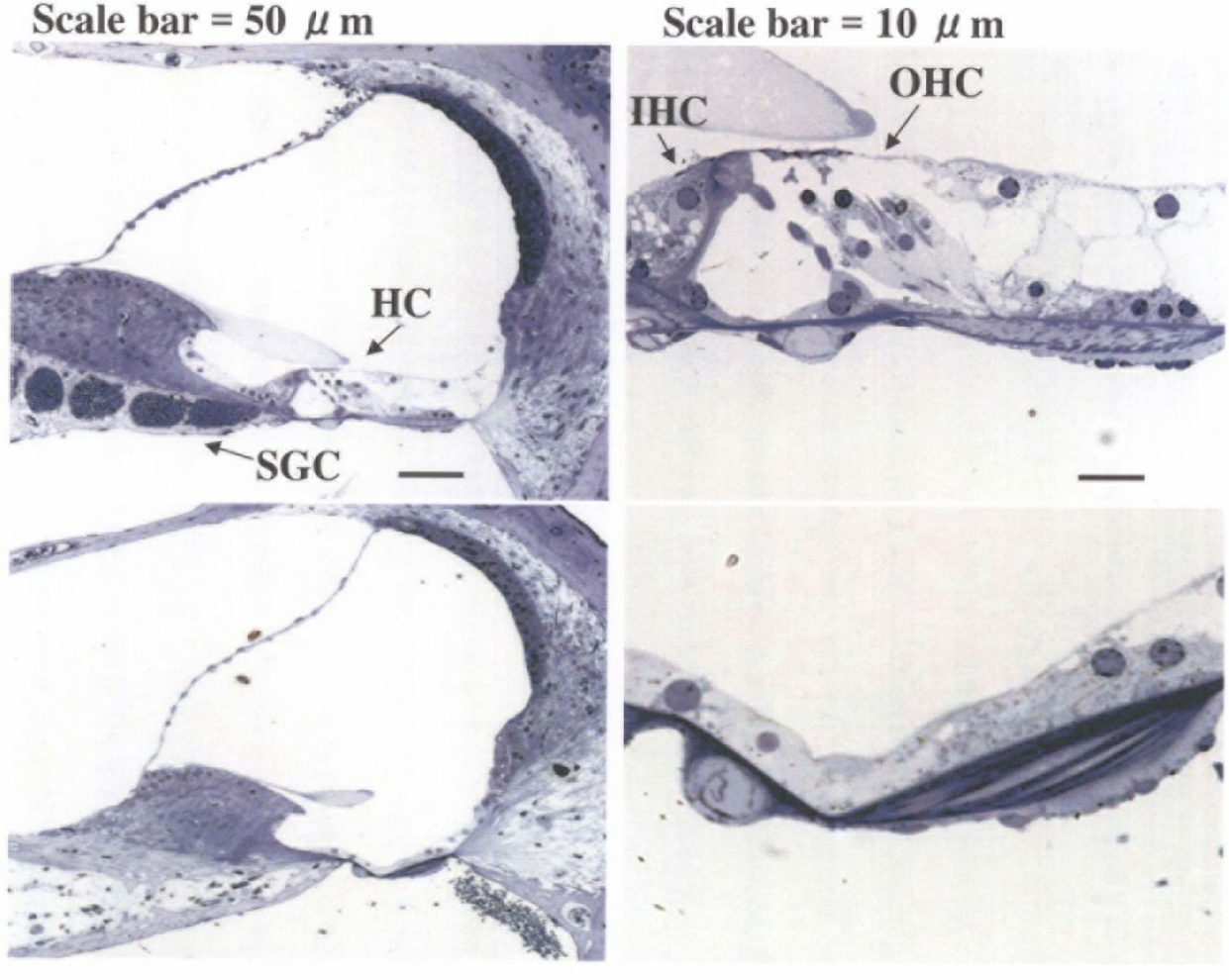

ABR Threshold (dB SPL)
0
10
20
30
40
50
60
70
80
0
5
10
15
20
Frequency (kHz)
control B6
CR B6

Figure 5-3. Comparison of Cochleae. Representative light micrographs of the lower basal cochlear turn from the 15-month-old control C57BL/6 (B6) mouse and the 15-month-old CR B6 mouse. The control B6 mouse exhibited severe loss of outer hair cells (OHC), inner hair cells (IHC), and supporting cells in the organ of Corti, and severe loss of spiral ganglion cells (SGC) in the cochlea, whereas the CR B6 mouse showed no degeneration of the organ of Corti, and spiral ganglion cells in the cochlea.

CR

Control


Scale bar = 50 μ m
Scale bar = 10 μ m
IHC
OHC
HC
SGC
Organ of Corti Lower base
Organ of Corti Lower base

associated with significant alterations in mRNA levels. Of the 45,037 genes and ESTs surveyed in the oligonucleotide arrays, we identified 164 genes up-regulated by CR and 48 genes down-regulated by CR (Table 5-1). The gene expression profile results revealed that AHL-related changes in the gene expression were remarkably reversed by CR. CR induced 17 hearing-related genes, including solute carrier family 1 (glial high affinity glutamate transporter), member 3 (*Slc1a3*), myosin 1B (*Myo1b*), gap junction membrane channel protein beta 6 (*Gjb6*), gap junction membrane channel protein beta 2 (*Gjb2*), otospiralin (*Otos*), and coagulation factor C homolog (*Limulus Polyphemus*) (*Coch*). These results were consistent with those of the ABR and the histology analysis. The gene with the largest upregulation in this transcriptional class was Slc1a3 gene (fold change = 9.5). Slc1a3 protein is a glutamate transporter highly expressed in the cochlea, and plays an important role in neurotransmission for afferent synapses in the peripheral auditory system (Kanai and Hediger, 2004; Hakuba *et al.*, 2000). Slc1a3-deficient mice show increased accumulation of glutamate in perilymphs, resulting in hearing loss. Myo1b gene is expressed at apical surfaces of Deiter's cells, the supporting cells which surround hair cells in the cochlea (Dumont *et al.*, 2002; Sherr *et al.*, 1993). Myo1b protein is a calmodulin- and actin-associated molecular motor, and may mediate adaptation of mechanoelectrical transduction in response to sound (Dumont *et al.*, 2002; Sherr *et al.*, 1993; Avraham *et al.*, 1995)). Gjb6 gene is expressed in supporting cells in the organ of Corti. Gjb6 protein is a member of connexins involved in formation of gap junctions between cells (Forge *et al.*,

2003; Kelley *et al.*, 1999). Mutations in this gene are thought to cause non-syndromic autosomal recessive deafness (Forge *et al.*, 2003; Kelley *et al.*, 1999). Gjb2 protein is also a member of connexins involved in formation of gap junctions between cells (Liu *et al.*, 2001). Gjb2 gene is also expressed in supporting cells in the organ of Corti, and mutations in human GJB2, which is homologous to mouse Gjb2, are responsible for the autosomal recessive isolated deafness, DFNB1 (Frenz and Van De Water, 2000; Cohen-Salmon *et al.*, 2002; Kelsell *et al.*, 1997). Otos gene is expressed in glical cells surrounding the spiral ganglion cells in the cochlea, and Otos protein is essential for survival of neurosensory epithelium (Delprat *et al.*, 2002). Downregulation of this gene are thought to causes hair cells degeneration and deafness (Delprat *et al.*, 2002). Coch gene is highly expressed in the cochlea, and Coch protein may play a structural role in the extracellular matrix in the cochlea (Robertson *et al.*, 1998; Yvette *et al.*, 1999). Mutations in human COCH, which is homologous to mouse Coch, are considered to cause non-syndromic, progressive hearing loss (Robertson *et al.*, 1998; Yvette *et al.*, 1999). Eighteen genes involved in energy metabolism, including solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5 (*Slc25a5*), ATPase, H+ transporting, VI subunit A, isoform 1 (*Atp6v1a1*), ATPase, H+ transporting, VI subunit D (*Atp6v1d*), cytochrome c oxidase, subunit VIa, polypeptide 1 (Cox6a1), and sirtuin 1 ((silent mating type information regulation 2, homolog) 1 (S. cerevisiae) (*Sirt1*) also displayed an increase in the gene expression. These results correlate well with the studies of calorie restriction on energy metabolism, which show that

CR may induce increased energy metabolism (Lin et al., 2002; Koubova and Guarente; 2003). The gene with the largest upregulation in this transcriptional class was *Slc25a5* gene (fold change = 2.3). Slc25a5 protein is involved in adenine nucleotide translocation in mitochondria (Cozens et al., 1989). Atp6v1a1 protein is involved in ATP biosynthesis in mitochondria (Mootha *et al.*, 2003), and Cox6a1 protein is involved in cytochrome c oxidase activity in the mitochondria (Mootha *et al.*, 2003). CR also induced the gene expression of Sirt1 gene. CR extends lifespan in numerous species (Lee *et al.*, 2002; Lee *et al.*, 1999; Lee *et al.*, 1999; Prolla, 2002). In yeast, the *SIR2* gene mediates the life-extending effects of CR (Lin *et al.*, 2002; Vaziri *et al.*, 2001; Tissenbaum *et al.*, 2001; Gasser *et al.*, 2001; Lin *et al.*, 2000; Kenyon, 2001). The mammalian *SIR2* orthologue, *Sirt1* also mediates cell survival in mammals, and Sirt1 protein represses p53-dependent apoptosis in response to DNA damage in mammals (Koubova *et al.*, 2003; Luo *et al.*, 2001; Picard *et al.*, 2004). Thus, this result suggests that CR retards the progression of AHL by induction of Sirt1 gene.

CR lowered the gene expression of 8 apoptosis-related genes, such as interleukin 6 (Il6), transformed mouse 3T3 cell double minute 2 (Mdm2), and Fas-associated factor 1 (Faf1). These results were consistent with the histological findings. These results also correlate well with the studies of apoptosis and AHL, which show that apoptosis may be associated with AHL (Fischel-Ghodsian, 1999). The gene with the largest downregulation in this transcriptional class was Il6 gene (fold change = -16.8). Il6 protein is a

proinflammatory cytokine, and Il6 may induce apoptosis in human neutrohils (Afford *et al.*, 1992; Margulies and Sehgal, 1993). Mdm2 is an oncoprotein that controls apoptosis through p53-dependent mechanisms (Prives and Hall, 1999; Vogelstein *et al.*, 2000). Mdm2 protein binds to p53, inactivates the ability of p53 to function as a transcription factor, and induces apoptosis (Prives and Hall, 1999; Vogelstein *et al.*, 2000). Mdm2 mRNA level is transcriptinally regulated by p53 in response to stress, such as DNA damage, and Mdm2 mRNA level is dependent of p53 (Prives and Hall, 1999; Chang *et al.*, 2004). Therefore, reduced p53 activity should lead to reduced Mdm2 mRNA level (Chang *et al.*, 2004). Fas, a member of the tumor necrosis factor receptor family, can induce apoptosis when activated by Fas ligand binding or anti-Fas antibody crosslinking. A Fas-associated protein factor, FAF1, potentiates Fas-mediated apoptosis (Chu *et al.*, 1995). CR lowered the gene expression of seven genes involved in stress response, such as solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2 (*Slc6a2*), thyroid peroxidase (*Tpo*), and trefoil factor 1 (Tff1). The norepinephrine (NE) transporter, Slc6a2 protein, regulates noradrenergic neurotransmission by efficiently clearing NE from synaptic spaces after release, and is involved in pain response (Fritz *et al.*, 1998). Tpo enzyme is involved in response to oxidative stress, a state often resulting from exposure to high levels of reactive oxygen species (Isozaki *et al.*, 1989). Tff1 protein is involved in response to wounding as a result of damage in the cochlea (Otto and Patel, 1999). CR also lowered the gene expression of 22 inflammatory response genes, such as interleukin 9 (*Il9*) and interleukin 1 receptor

Table 5-1. List of selected genes altered in the expression by CR. This table lists genes of selected classes that were significantly (*P* value = 0.01 >, FC = 1.2 ≦) altered in gene expression with CR. 45,037 genes and ESTs were screened using GeneChips (Mouse Genome 430 2.0). The fold change shown represents the average of all nine possible pairwise comparisons among individual samples (n = 3 for each group, two mice were used for one GeneChip) determined using the Microarray Suit Expression Analysis software. GenBank accession numbers are listed under Gene ID.

Gene ID	FC	Gene
Perception of Sound		
BB357585	9.5	solute carrier family 1 (glial high affinity glutamate transporter), member 3
AA406997	6.4	myosin IB
NM_007583	5.0	calcium channel, voltage-dependent, gamma subunit 2
BC016507	2.9	gap junction membrane channel protein beta 6
AF322631	2.6	potassium inwardly-rectifying channel, subfamily J, member 10
AV239646	2.4	gap junction membrane channel protein beta 2
AY078071	2.3	otospiralin
NM_008770	2.3	claudin 11
BB731671	2.2	coagulation factor C homolog (Limulus polyphemus)
M63801	2.1	gap junction membrane channel protein alpha 1
BG144467	2.0	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)
BM119623	1.9	activating transcription factor 2
NM_007644	1.9	scavenger receptor class B, member 2
BE994609	1.4	myelin basic protein
AF090403	1.4	quaking
AF177196	1.4	deiodinase, iodothyronine, type II
NM_025567	1.3	cytochrome c-1
Energy Metabolism		
AV110784	2.3	solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5
BE914497	2.1	phosphofructokinase, liver, B-type

(*continued*)

Gene ID	FC	Gene
AV336908	1.9	dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)
NM_007508	1.8	ATPase, H+ transporting, V1 subunit A, isoform 1
AV105788	1.8	ATPase, H+ transporting, V1 subunit D
BB549292	1.7	monoamine oxidase B
NM_022433	1.7	sirtuin 3 (silent mating type information regulation 2, homolog) 3 (S. cerevisiae)
NM_007748	1.7	cytochrome c oxidase, subunit VI a, polypeptide 1
AA987147	1.6	ATPase, H+ transporting, V1 subunit C, isoform 1
NM_007750	1.6	cytochrome c oxidase, subunit VIIIa
NM_008492	1.6	lactate dehydrogenase 2, B chain
AK019085	1.5	ubiquinol-cytochrome c reductase hinge protein
AK005273	1.4	succinate-Coenzyme A ligase, ADP-forming, beta subunit
NM_019913	1.4	thioredoxin 2
AF354051	1.4	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d
AV124743	1.4	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2
NM_009941	1.3	cytochrome c oxidase subunit IV isoform 1
BC027270	1.3	NADH dehydrogenase (ubiquinone) Fe-S protein 3
BB713410	1.3	hexokinase 1
NM_019812	1.3	sirtuin 1 ((silent mating type information regulation 2, homolog) 1 (S. cerevisiae)
Neurotransmission/Neuronal Factors		
BF462185	10.2	4-aminobutyrate aminotransferase
NM_010298	3.0	glycine receptor, beta subunit
BQ268470	2.8	gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 1
NM_013540	2.7	glutamate receptor, ionotropic, AMPA2 (alpha 2)

(*continued*)

Gene ID	FC	Gene
NM_008989	2.2	purine rich element binding protein A
BC025807	2.1	ATPase, Na+/K+ transporting, alpha 2 polypeptide
BE947704	1.9	meteorin
NM_025292	1.8	synaptojanin 2 binding protein
BB648600	1.8	reticulon 4
BB825002	1.6	solute carrier family 39 (metal ion transporter), member 6
NM_007744	1.5	catechol-O-methyltransferase
BC018383	1.5	internexin neuronal intermediate filament protein, alpha
BQ175666	1.5	gamma-aminobutyric acid (GABA-A) receptor, subunit beta 3
Apoptosis		
NM_031168	-16.8	interleukin 6
BB459486	-5.2	transformed mouse 3T3 cell double minute 2
AF244979	-2.4	lectin, galactose binding, soluble 12
AV016515	-2.2	Fas-associated factor 1
BC003750	-2.1	transformed mouse 3T3 cell double minute 4
BB284358	-1.9	EGL nine homolog 3 (C. elegans)
NM_007545	-1.8	BH3 interacting (with BCL2 family) domain, apoptosis agonist
BM119782	-1.5	B-cell leukemia/lymphoma 2

(*continued*)

Gene ID	FC	Gene
Stress Response		
BE956505	-4.4	solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2
AI561924	-4.1	RIKEN cDNA A030014E15 gene
NM_009417	-3.7	thyroid peroxidase
NM_027844	-3.1	RIKEN cDNA 4833428E21 gene
NM_009362	-2.0	trefoil factor 1
BB044157	-1.8	c6.1a protein
BB215260	-1.4	bleomycin hydrolase
Inflammatory Response/Immune Modulation		
NM_008373	-7.8	interleukin 9
BE285634	-3.2	interleukin 1 receptor accessory protein
BB821318	-3.0	inhibitor of kappaB kinase gamma
NM_008533	-2.9	lymphocyte antigen 78
BB558275	-2.9	phosphatidylinositol 3-kinase catalytic delta polypeptide
D16220	-2.7	CD80 antigen
BB667651	-2.7	pyruvate kinase liver and red blood cell
AV252273	-2.5	interleukin 4 receptor, alpha
AF285585	-2.5	ring finger protein 17
NM_019494	-2.5	chemokine (C-X-C motif) ligand 11
AV276986	-2.4	Casitas B-lineage lymphoma b
M57525	-2.4	interleukin 1 receptor antagonist

(*continued*)

Gene ID	FC	Gene
AF206697	-2.3	interleukin 1 family, member 6
BB154331	-2.3	CD8 antigen, alpha chain
BM224327	-2.2	Fc receptor, IgG, low affinity IIb
BC019961	-2.0	chemokine (C-X-C motif) ligand 16
NM_008920	-1.9	proteoglycan 2, bone marrow
U66888	-1.9	EGF-like module containing, mucin-like, hormone receptor-like sequence 1
AV231648	-1.6	chemokine (C-C motif) receptor 1
AW986054	-1.6	interferon-induced protein 35
BB148128	-1.5	chemokine (C-C motif) receptor 2
NM_009841	-1.3	CD14 antigen
Proteolysis and peptidolysis/Catabolism		
NM_007919	-8.8	elastase 2
NM_022326	-6.1	cathepsin M
NM_009390	-5.0	tolloid-like
AK010153	-4.7	titin
D17583	-4.0	proprotein convertase subtilisin/kexin type 5
U42405	-2.8	mast cell protease 7
NM_019429	-2.7	protease, serine, 16 (thymus)
BM935152	-2.2	ring finger protein 130
BB535404	-2.1	matrix metalloproteinase 14 (membrane-inserted)
AV023994	-2.1	cathepsin L

(*continued*)

Gene ID	FC	Gene
AI875733	-1.7	nardilysin, N-arginine dibasic convertase, NRD convertase 1
Ion Transport		
BE655147	7.5	solute carrier family 4 (anion exchanger), member 4
NM_011596	7.0	ATPase, H+ transporting, lysosomal V0 subunit a isoform 2
BF660388	6.4	protein kinase C, beta 1
AV152334	2.8	ATPase, Na+/K+ transporting, beta 1 polypeptide
BB667135	2.6	solute carrier organic anion transporter family, member 1c1
NM_010585	2.6	inositol 1,4,5-triphosphate receptor 1
BB321232	2.3	potassium channel tetramerisation domain containing 3
AV320241	2.1	transient receptor potential cation channel, subfamily M, member 7
AV361923	2.1	potassium voltage-gated channel, shaker-related subfamily, member 1
BB332449	2.1	ceruloplasmin
BB560955	1.8	ATPase, H+ transporting, lysosomal accessory protein 2
BC017615	1.5	solute carrier family 24 (sodium/potassium/calcium exchanger), member 3
BB249222	1.5	polycystic kidney disease 2
NM_025272	1.4	ATPase, H+ transporting, V0 subunit
Muscle Contraction/Muscle Modulation		
BI248947	2.5	caldesmon 1
AK017400	2.0	dystrobrevin binding protein 1
AA245637	1.9	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
AV172168	1.6	calponin 3, acidic

(*continued*)

Gene ID	FC	Gene
Structure/Structural Modulation		
BB239540	5.6	Down syndrome cell adhesion molecule-like 1
AF427498	3.6	cadherin EGF LAG seven-pass G-type receptor 3
BC015076	2.8	epithelial V-like antigen 1
NM_009864	1.8	cadherin 1
AI448973	1.8	calsyntenin 2
NM_011707	1.7	vitronectin
NM_016898	1.7	CD164 antigen
BC020531	1.7	spondin 1, (f-spondin) extracellular matrix protein
NM_019410	1.7	profilin 2
NM_011995	1.6	piccolo (presynaptic cytomatrix protein)
AI385532	1.6	thrombospondin 1
BB534670	1.5	CD36 antigen
NM_009818	1.4	catenin alpha 1
NM_010878	1.3	non-catalytic region of tyrosine kinase adaptor protein 1
BB775640	1.2	catenin src
Growth Factors/Development		
NM_054077	9.5	proline arginine-rich end leucine-rich repeat
BC002064	8.5	pleiotrophin
BI134907	7.6	catenin beta
AW536452	5.4	cullin 4B

(*continued*)

Gene ID	FC	Gene
BC024400	5.3	coiled-coil domain containing 5
NM_021465	4.5	stromal antigen 2
BF322051	4.1	anaphase promoting complex subunit 10
AW912678	3.5	abl-interactor 1
NM_007589	3.4	calmodulin 2
W41916	3.1	guanine nucleotide binding protein, alpha q polypeptide
BE631549	2.4	N-myc downstream regulated gene 3
NM_010882	2.4	necdin
AW111876	2.2	phosphatidic acid phosphatase type 2B
BI250645	2.2	pogo transposable element with ZNF domain
AF215668	2.0	regulator of G-protein signaling 2
BE824561	1.9	cell division cycle 37 homolog (S. cerevisiae)-like 1
BC013545	1.9	centrin 2
AV015462	1.7	calmodulin 1
NM_008179	1.6	G1 to phase transition 2
AU018448	1.6	pituitary tumor-transforming 1 interacting protein
AK007630	1.6	cyclin-dependent kinase inhibitor 1A (P21)
BC003220	1.6	E2F transcription factor 5
BC026606	1.5	CDC16 cell division cycle 16 homolog (S. cerevisiae)
NM_010514	1.5	insulin-like growth factor 2
BC003856	1.4	protein phosphatase 2a, catalytic subunit, alpha isoform

(*continued*)

Gene ID	FC	Gene
AK009373	1.3	cell division cycle 2-like 5 (cholinesterase-related cell division controller)
AU042749	1.3	mortality factor 4 like 1
NM_011666	1.3	ubiquitin-activating enzyme E1C
BM227770	1.3	calcium/calmodulin -dependent protein kinase II gamma
AA124924	1.3	RAS p21 protein activator 1
Biosynthesis		
M94967	3.3	prostaglandin-endoperoxide synthase 2
NM_023627	3.2	myo-inositol 1-phosphate synthase A1
BB314559	2.2	pumilio 1 (Drosophila)
AK017631	2.2	nicotinamide nucleotide adenylyltransferase 1
BG072903	2.1	C-terminal binding protein 2
NM_010282	2.0	geranylgeranyl diphosphate synthase 1
AV212753	1.6	asparagine synthetase
BB221333	1.5	quininoid dihydropteridine reductase
AK003237	1.4	vitamin K epoxide reductase complex, subunit 1
BI689507	1.3	pumilio 2 (Drosophila)
DNA Synthesis/DNA Repair		
NM_008893	2.7	polymerase (DNA directed), alpha 2
BM196962	2.2	checkpoint supressor 1
BF161073	1.8	origin recognition complex, subunit 3-like (S. cerevisiae)
BG868960	1.7	ubiquitin-conjugating enzyme E2A, RAD6 homolog (S. cerevisiae)

(*continued*)

Gene ID	FC	Gene
BM209585	1.6	SMT3 suppressor of mif two 3 homolog 1 (yeast)
BM229104	1.5	SET translocation
Y07687	1.3	nuclear factor I/B
AK005647	1.3	chondroitin sulfate proteoglycan 6
Protein Synthesis/Modification		
BG071958	6.5	ribosomal protein L7-like 1
AK004612	5.6	GTP binding protein 1
NM_022891	4.6	ribosomal protein L23
W11855	3.8	ribosomal protein L14
NM_009455	2.4	ubiquitin-conjugating enzyme E2E 1, UBC4/5 homolog (yeast)
BM114165	2.2	ribosomal protein L5
BM117243	2.2	RIKEN cDNA 5330414D10 gene
BB791850	2.2	ubiquitin-like 1 (sentrin) activating enzyme E1B
NM_019912	2.0	ubiquitin-conjugating enzyme E2D 2
BE308547	1.9	RIKEN cDNA 5730427N09 gene
NM_053161	1.9	mitochondrial ribosomal protein L27
BM240314	1.8	eukaryotic translation initiation factor 4A2
BQ179556	1.7	mitochondrial ribosomal protein L17
AW701127	1.7	eukaryotic translation initiation factor 3, subunit 10 (theta)
BC004614	1.6	mitochondrial ribosomal protein L30
AV035110	1.6	mitochondrial ribosomal protein S6

(*continued*)

Gene ID	FC	Gene
NM_018799	1.6	eukaryotic translation initiation factor 3, subunit 2 (beta)
BC013717	1.6	eukaryotic translation termination factor 1
BF228007	1.5	ribosomal protein L17 /// Similar to Rpl17 protein (LOC384641), mRNA
BC003856	1.4	protein phosphatase 2a, catalytic subunit, alpha isoform
AV139821	1.4	Tax1 (human T-cell leukemia virus type I) binding protein 3 /// ribosomal protein L13
BG066549	1.4	ubiquitin-conjugating enzyme E2L 3
BC021304	1.3	mitochondrial ribosomal protein L15
BC006682	1.3	eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked
BG069767	1.3	ribosomal protein S3
BI904160	1.3	RIKEN cDNA 1200009C21 gene
BM936366	1.3	ubiquitin specific protease 3
AK013880	1.3	asparaginyl-tRNA synthetase
NM_009079	1.2	ribosomal protein L22
AI594880	1.2	mitochondrial ribosomal protein L51
BC019372	1.2	ubiquitin-conjugating enzyme E2 variant 1
NM_026533	1.2	ribosomal protein S13
AK018443	1.2	ubiquitin protein ligase E3A
AK009324	1.2	ubiquitin-conjugating enzyme E2Q (putative)
Fatty Acid Metabolism		
NM_023737	2.5	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase
BC013442	2.3	solute carrier family 27 (fatty acid transporter), member 2

(*continued*)

Gene ID	FC	Gene
AK012088	2.3	acyl-CoA synthetase long-chain family member 3
NM_009949	1.8	carnitine palmitoyltransferase 2
NM_008212	1.5	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain
BC006692	1.5	acyl-CoA synthetase long-chain family member 1
NM_025797	1.5	cytochrome b-5
AK010307	1.4	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1
BG074607	1.4	adiponectin receptor 2

accessory protein (Il1rap), which are involved in cytokine activity (Erpenbeck *et al.*, 2003).

CR induced the gene expression of 11 proteolysis-related genes, including elastase 2 and cathepsin M. Elastase 2, or neutrophil elastase (NE) is a potent serine protease, and is required for maximal intracellular killing of Gram negative bacteria by neutrophils (Belaaouaj *et al.*, 1998). CR induced the gene expression of 13 neurotransmission-related genes, including 4-aminobutyrate aminotransferase (*Abat*) and gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 1 (*Gabra1*). Gabra1 is involved in neurotransmitter GABA-A activity, and functions as chloride channels (Buckle *et al.*, 1989). CR induced the gene expression of 14 ion transport-related genes including solute carrier family 4 (anion exchanger), member 4 and ATPase, H^+ transporting, lysosomal V0 subunit a isoform2. CR also induced transcripts associated with muscle contraction, such as caldesmon 1 and calponin 3, acidic. CR was characterized by induction of structural modulation, growth factor, biosynthesis, DNA synthesis, DNA repair, protein synthesis, and fatty acid metabolism.

Summary of findings

ABR results show that CR retards the progression of AHL. Histology results also show CR prevented degeneration of the organ of Corti in the CR cochlea, supporting the ABR finding. The gene expression profile results revealed that AHL-related changes in the gene expression were remarkably

reversed by CR. A summary of global view of transcriptional changes induced by CR is shown in Table 5-1. These data indicate that CR induced hearing-related genes and energy metabolism-related gene such as *Sirt1*. CR also lowered the gene expression of apoptosis-related genes such as *Mdm2* and *Il6* which mediate p53-dependent apoptosis (Prives and Hall, 1999; Vogelstein *et al.*, 2000; Afford *et al.*, 1992; Margulies and Sehgal, 1993), and stress response. CR extends lifespan in numerous species (Lee *et al.*, 2002; Lee *et al.*, 1999; Lee *et al.*, 1999; Prolla, 2002; Drew *et al.*, 2002). In yeast, the *SIR2* gene mediates the life-extending effects of CR (Lin *et al.*, 2002; Vaziri *et al.*, 2001; Tissenbaum *et al.*, 2001; Gasser *et al.*, 2001; Lin *et al.*, 2000). The mammalian *SIR2* orthologue, *Sirt1* also mediates cell survival in mammals, and Sirt1 protein represses p53-dependent apoptosis in response to DNA damage in mammals (Koubova *et al.*, 2003; Luo *et al.*, 2001; Picard *et al.*, 2004). Thus, taken together, these suggest that CR retards the progression of AHL by mechanisms that may be related to induction of energy metabolism-related genes such as *Sirt1*, and suppression of apoptosis.

Table 5-2. Global view of transcriptional changes induced by CR. This table lists selected classes of transcriptional changes induced by CR.

Function	FC	Description
Perception of Sound	↑	Induction of hearing-related genes
Energy metabolism	↑	Increased energy metabolism
Neurotransmission/Neuronal Factors	↑	Increased neurotransmitter transport, neurogenesis
Apoptosis	↓	Suppression of apoptosis
Stress Response	↓	Supprssion of oxidative stress-inducible genes
Inflammatory Response	↓	Suppression of inflammatory response, immune response
Proteolysis and Peptidolysis	↓	Decreased proteolysis and peptidolysis
Ion Transport	↑	Increased potassium ion transport, sodium ion transport,
Muscle Contraction/Muscle Modulation	↑	Induction of muscle contraction and muscle development
Structure/Structural Modulation	↑	Induction of cytoskeleton organization and cell adhesion
Growth Factors/Development	↑	Induction of cell growth and cell differentiation
Biosynthesis	↑	Increased glycolipid biosynthesis and prostaglandin biosynthesis
DNA Synthesis/DNA Repair	↑	Induction of DNA replication factors and DNA repair factors
Protein Synthesis/Modification	↑	Increased protein biosynthesis and protein modification
Fatty Acid Metabolism	↑	Increased fatty acid biosynthesis