Mclk1+/- mice are not resistant to the development of atherosclerosis
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Abstract
Background: Mice with a single copy of Mclk1 (a.k.a. Coq7), a gene that encodes a mitochondrial enzyme required for the biosynthesis of ubiquinone and other functions, live longer than wild-type mice. The prolonged survival implies a decreased mortality from age-dependent lethal pathologies. Atherosclerosis is one of the main age-dependent pathologies in humans and can be modeled in mice that lack Apolipoprotein E (ApoE−/-) or mice that lack the Low Density Lipoprotein Receptor (LDLr−/-) in addition to being fed an atherosclerosis-inducing diet. We sought to determine if Mclk1 heterozygosity protects against atherosclerosis and dyslipidemia in these models.

Results: We found that Mclk1 heterozygosity did not protect against dyslipidemia, oxidative stress, or atherosclerosis in young (6 or 10 months) or older (18 months) mice. Furthermore, the absence of ApoE suppressed the lifespan-promoting effects of Mclk1 heterozygosity.

Conclusion: These findings indicate that although Mclk1 heterozygosity can extend lifespan of mice, it does not necessarily protect against atherosclerosis. Moreover, in the presence of hyperlipidemia and chronic inflammation, Mclk1 heterozygosity is incapable of extending lifespan.

Introduction
Median lifespan is increased by mutations in the gene clk-1, and by heterozygosity for a null allele of the mammalian clk-1 homologue Mclk1, in Caenorhabditis elegans and mice, respectively [1-3]. CLK-1/MCLK1 is a mitochondrial hydroxylase required for the biosynthesis of ubiquinone (UQ or Coenzyme Q) [4,5], a lipid-like molecule that plays a vital role as an electron transporter in the mitochondrial electron transport chain, is an important lipid-soluble antioxidant, and also performs a variety of other functions [6]. Mclk1-null (Mclk1−/-) cells and embryos make no UQ and the embryos are not viable, while Mclk1 heterozygous (Mclk1+/−) cells and mice are superficially normal and show normal levels of UQ [7,8]. While the biochemical phenotypes associated with Mclk1 heterozygosity have begun to be uncovered [3,9], the actual effect on the mouse in terms of resistance to the physical decline associated with age and to age-dependent disease has not yet been examined.

The longest-lived humans display an increased resistance to the common age-dependent diseases (cardiovascular disease, stroke, cancer, etc.), as the majority of them either develop these diseases later than the general population or not at all [10,11]. It is therefore tempting to expect a similar resistance to the development of age-dependent diseases in Mclk1+/- and other lines of long-lived mice. One particularly prevalent age-dependent disease is atherosclerosis, in which the oxidative modification of low-density lipoprotein and the resultant recognition by
cells of the immune system results in a chronic inflammatory state within the wall of blood vessels. This leads to the formation of a plaque containing a mixture of extracellular cholesterol deposits, immune cells and infiltrating smooth muscle cells [12]. Interestingly, manipulations that extend lifespan in mice have been shown to protect against atherosclerosis. Among long-lived mice, calorically restricted mice as well as genetically modified p66shc-/- and UCP1 transgenic mice have been evaluated for atherosclerosis susceptibility. The fact that both were found to have an increased resistance to the disease [13-15] suggests that Mclk1+/− mice may also be resistant to the disease.

A further incentive for studying the effect of Mclk1 heterozygosity on atherosclerosis is the finding that clk-1 worms have an altered lipid metabolism, consistent with a decrease in oxidatively modified lipoproteins [16]. Furthermore, the core non-lipid component of these particles appears to be vitellogenin, a protein that is homologous to apolipoprotein B, the main structural component of atherosclerosis-inducing lipoprotein particles in mammals [17,18]. The conservation of lipid metabolism between worms and mammals was further confirmed by the finding that several lipid-lowering drugs developed in mammals are active on lipoprotein metabolism in worms [19].

Atherosclerosis does not appear to develop in mice fed a regular diet, due to the partitioning of most cholesterol into the anti-atherogenic High Density Lipoprotein fraction of lipoproteins. Therefore, the lifespan extending effect of Mclk1 heterozygosity cannot be due to a decrease in atherosclerosis-related mortality. However, the underlying conditions thought to be responsible for atherosclerosis, oxidative stress and inflammation, are known to be strongly associated with aging, justifying the study of atherosclerosis in aging mice [20,21]. Mice lacking the Low Density Lipoprotein Receptor and fed a high-fat, high-cholesterol "Western" diet (LDLr-/-), as well as mice lacking apolipoprotein E fed a regular chow diet (ApoE-/-), develop hyperlipidemia and extensive aortic atherosclerosis, and are commonly used to model atherosclerosis in mice [22,23]. Although the lipid profile of these mice is characteristic of only the more dyslipidemic humans, the histopathological characteristics of the lesions appear similar to those observed in humans and other models, even if they develop over a shorter timeframe [24]. Furthermore, although coronary artery disease predominately affects those of middle-age or older, the atherosclerotic lesions that underlie the disease (and which are the focus of this study) begin to develop much earlier in life, with young adults having comparable aortic atherosclerosis to that observed in mice at an equivalent stage in their lives [25].

We elected to use two separate models because, although they theoretically affect the same system (the uptake of lipid from the circulation), they are known to respond differently to certain interventions [26-28]. Importantly, the LDLr-/- model develops more severe atherosclerosis than the ApoE-/- model, allowing us to measure the effect of Mclk1 heterozygosity on atherosclerosis of both medium and high severity. We also measured atherosclerosis in mice of different ages, in order to detect any possible impact of an age-dependent effect of Mclk1 heterozygosity on atherosclerosis. Here, we demonstrate that Mclk1 heterozygosity does not consistently affect atherosclerosis or lipid profile in either model. Furthermore, Mclk1 heterozygosity fails to extend lifespan in atherosclerosis-susceptible ApoE-/- mice.

**Methods**

**Animals and Diet**

LDLr-/- [29] and ApoE-/- [30] mice on a C57Bl/6J background were purchased from The Jackson Laboratory (Bar Harbor, ME) and were crossed to Mclk1+/- mice, previously produced by gene targeting [7], to produce LDLr-/-;Mclk1+/-, LDLr-/-;Mclk1+/-; apoE-/-; Mclk1+/- and ApoE-/-; Mclk1+/- animals. Genotypes were determined by PCR. Mice were maintained on 4.5% fat rodent chow (Charles River diet 5075). At 3 months of age in the LDLr-/- groups were placed on a "Western"-type diet (Harlan Teklad, Madison, WI, TD.01444) containing 21% (w/w) anhydrous milk fat and 0.15% cholesterol. Mice in the ApoE-/- groups were fed regular rodent chow throughout the experiments. Mice were housed in a specific pathogen free facility at McGill University, 2–5 animals per cage, and fasted overnight prior to sacrifice by anesthetic overdose (Ketamine/Xylazine/Acepromazine).

ApoE-/- mice were sacrificed at 10 and 18 months of age and LDLr-/- mice at 6 and 10 months of age (Table 1). 18 months was judged the greatest age that we could obtain without significant losses due to mortality in the ApoE-/- group. We had originally intended to sacrifice the LDLr-/- mice at 18 months as well, but we found that after 10 months of age on the "Western" diet LDLr-/- mice developed severe health problems. The earlier measurement point for each model is the youngest age where we could...

**Table 1: Experimental Design**

| Model     | Age (months) | Gender(s) | Background (# Backcrosses) |
|-----------|--------------|-----------|-----------------------------|
| ApoE-/-   | 10           | M, F      | CBA × C57BL/6j (4)          |
|           | 10           | M, F      | 1295v/BaIb/c × C57BL/6j (10) |
|           | 18           | F         | CBA × C57BL/6j (3)          |
| Lifespan  | M, F         | 1295v/BaIb/c × C57BL/6j (6) |
| LDLr-/-   | 6            | M, F      | CBA × C57BL/6j (3)          |
|           | 10           | F         | 1295v/BaIb/c × C57BL/6j (6) |

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reliably obtain measurable atherosclerosis in each case. For both groups, male and female mice were sacrificed at the earlier time-point, and females only at the later time-point.

The genetic backgrounds of the mice varied somewhat between studies, although they were always consistent within a study (Table 1). Mice on the CBA background were backcrossed 3 times into the C57BL/6J background to create the LDLr/- mice sacrificed at 6 months of age and mice on a mixed 129Sv × Balb/c background were backcrossed 6 times for LDLr/- mice sacrificed at 10 months of age. ApoE/- mice sacrificed at 10 and 18 months were backcrossed 4 and 3 times respectively out of the CBA background, and those used in the aging experiment were backcrossed 6 times out of a mixed 129Sv × Balb/c background. A second group of ApoE/- mice sacrificed at 10 months of age was backcrossed 10 times from the same background. In all cases, the C57BL/6J strain made the greatest contribution to the background, which is important because of this strain’s relative sensitivity to atherosclerosis development [31], as well as because the ability of Mclk1 heterozygosity to extend lifespan in this strain has been confirmed in our lab [32].

To determine the lifespan of Mclk1+/ mice in the ApoE/+ background, mice were kept until either natural death, or evidence of impending mortality (such as sudden drastic weight loss, lack of movement or a severely distended abdomen) necessitating euthanasia.

All studies were approved by the McGill Faculty of Science Animal Care Committee and conducted according to the guidelines of the Canadian Council on Animal Care.

Atherosclerosis Severity
The surface area of atherosclerotic lesions was measured on the inner surface of the aorta, from the aortic origin to the iliac branch point, as has been previously described [33,34]. Quantification of staining on the acquired images was carried out using UTHSCSA ImageTool v3.0 (University of Texas Health Science Center in San Antonio, USA), and results were expressed as percentage surface area of the aorta occupied by Oil Red O-staining lesions. The innominate, common carotid and subclavian arteries were excluded from the analysis.

Plasma Measurements
EDTA plasma was collected by cardiac puncture of anaesthetized mice, flash-frozen in liquid nitrogen and stored at -80°C. Kits for measuring plasma cholesterol were obtained from Wako Chemicals USA and those for triglycerides from Sigma-Aldrich. Lipid peroxidation, as quantified by the level of malondialdehyde (MDA), was measured with a Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit from ZeptoMetrix Corporation. The TBARS method of measuring MDA has received considerable criticism for being an insufficiently representative measurement of lipid peroxidation [35-37]. Despite this, even some of the harshest critics concede that the assay frequently yields useful results supported by other better-validated assays, although it may be better thought of as a measurement of susceptibility to lipid peroxidation rather than the steady-state level of damage [37].

Statistics
The non-parametric Mann-Whitney test was used to compare atherosclerosis surface area in prepared aortas. Otherwise, the unpaired two-tailed Student’s t-test was used. Survival curves were compared using the Log-rank (Mantel-Cox) test. Two-way ANOVA with Bonferroni posttests was used for comparing body-weights collected over lifespan. Comparisons were always made between genotypes, within genders, using Prism 4.03 (GraphPad Software, Inc).

Results
Mclk1 heterozygosity does not decrease the severity of atherosclerosis
To determine if Mclk1 heterozygosity protected against atherosclerosis, Mclk1+/ mice were crossed into atherosclerosis-sensitive ApoE+/- and LDLr/- backgrounds. The proportion of the inner aortic surface occupied by atherosclerotic lesions was then quantified. Atherosclerosis in both the ApoE+/- and LDLr/- models of the disease was not inhibited by Mclk1 heterozygosity (Figures 1 and 2). In one condition (ApoE+/- mice sacrificed at 10 months of age), Mclk1+/ females had increased atherosclerosis (6.8 vs. 10 percent of aortic surface area, p = 0.0146). In the same experiment, there was a 37 percent decrease in atherosclerosis in Mclk1+/ males that was not statistically significant (p = 0.21). As the mice in this cohort had only been backcrossed for four generations, it is possible that high variability due to genetic heterogeneity could have been masking an effect of Mclk1 heterozygosity in males. We therefore repeated this study in a second group of ApoE+/- mice that had been backcrossed ten generations onto the C57BL/6J background. In this second cohort of mice, there was no effect of Mclk1 heterozygosity in either gender.

In addition, Mclk1 heterozygosity in these atherosclerosis susceptible backgrounds did not produce any consistent change in lipid profiles (Table 2), including total plasma cholesterol and triglycerides. Mclk1+/ ApoE+/- males sacrificed at 10 months of age had decreased plasma triglycerides. However, when this experiment was repeated in the fully congenic background, triglycerides were not affected. Instead, we saw an increase in cholesterol levels. Plasma lipid peroxidation was never significantly affected. Body
Heterozygosity for Mclk1 does not prevent atherosclerosis in the LDLr<sup>−/−</sup> disease model. Atherosclerotic lesions that stain with the lipid-sensitive dye Oil Red O were quantified on the inner surface of the aorta in LDLr<sup>−/−</sup> mice at (A) 6 months of age, with three backcrosses into C57BL/6J and (B) 10 months of age with six backcrosses into C57BL/6J. F and M labels stand for females and males, respectively. The +/+ and +/- labels stand for the Mclk1<sup>+/+</sup> and Mclk1<sup>+/−</sup> genotypes, respectively.

**Figure 1**

Heterozygosity for Mclk1 does not prevent atherosclerosis in the LDLr<sup>−/−</sup> disease model. Atherosclerotic lesions that stain with the lipid-sensitive dye Oil Red O were quantified on the inner surface of the aorta in LDLr<sup>−/−</sup> mice at (A) 6 months of age, with three backcrosses into C57BL/6J and (B) 10 months of age with six backcrosses into C57BL/6J. F and M labels stand for females and males, respectively. The +/+ and +/- labels stand for the Mclk1<sup>+/+</sup> and Mclk1<sup>+/−</sup> genotypes, respectively.
Heterozygosity for Mclkl does not prevent atherosclerosis in the ApoE<sup>-/-</sup> disease model. Atherosclerotic lesions that stain with the lipid-sensitive dye Oil Red O were quantified on the inner surface of the aorta in ApoE<sup>-/-</sup> mice at (A) 10 months, initial trial with four backcrosses into C57BL/6J, (B) 10 months, second trial with ten backcrosses into C57BL/6J and (C) 18 months, with three backcrosses into C57BL/6J. F and M labels stand for females and males, respectively. The ++/+ and +/- labels stand for the Mclkl<sup>++/+</sup> and Mclkl<sup>++/-</sup> genotypes, respectively.
weight of female LDLr−/−; Mclk1+/− mice sacrificed at six months of age was slightly decreased, but we did not see an effect on body weight in any other group.

**Loss of ApoE suppresses the lifespan extension conferred by Mclk1 heterozygosity**

Male and female Mclk1+/− mice on an ApoE-null background failed to live longer than Mclk1+/+ controls (Figure 3). Female Mclk1+/− mice actually had a shorter average lifespan than controls, although this difference did not reach statistical significance (p = 0.16). In Mclk1+/− mice of both genders, body weight was decreased throughout life (p < 0.0001 females, p = 0.0064 males) (Figure 3). 21 out of 35 females and 9 out of 18 males were euthanized as they were obviously near death due to severe illness (proportions were equal for each genotype), with the remainder dying naturally. Atherosclerosis was quantified in euthanized mice, and Mclk1+/− females appeared to have decreased atherosclerosis relative to controls, although this difference did not reach statistical significance (16.4 ± 12.5 in Mclk1+/− vs. 9.6 ± 5.8 percent in Mclk1+/+, p = 0.18 by Mann-Whitney test). Atherosclerosis was not affected in males (20.3 ± 14.25 in wild-type vs. 23 ± 16.57 in Mclk1+/−, p = 0.63). In the group of ApoE−/− mice sacrificed at 18 months of age, three mice of each genotype died prior to their date of sacrifice, closely paralleling the survival curve of mice in the lifespan study.

**Discussion**

**The effect of Mclk1 heterozygosity on atherosclerosis**

In five separate studies, we showed that Mclk1 heterozygosity did not ameliorate atherosclerosis in mice. In one study, female Mclk1+/− mice showed a statistically significant increase in atherosclerosis, and males showed a non-significant decrease. However, we were unable to repeat either of these results in a follow-up study in which the mice were in a fully congenic background. Our experiments covered a range of ages and severity of disease, indicating that Mclk1 heterozygosity did not have any beneficial age-dependent or independent effects on atherosclerosis. In other words, we only observed an effect on atherosclerosis in one particular genetic background, suggesting that it was due to a unique set of interactions within this particular background. This forces us to conclude that any potential effects of Mclk1 heterozygosity on atherosclerosis would not be due to the same mechanism as that which produces lifespan extension, which was observed in several separate backgrounds.

We have recently reported that young Mclk1+/− mice have decreased lipid peroxidation, measured via plasma isoprostanes. At the same time, the mice had substantial alterations in mitochondrial function, including a general decrease in mitochondrial respiration and an increase in mitochondrial oxidative stress [9]. Mitochondrial dysfunction linked to increased oxidative stress, such as that found in Mclk1+/− mice, has been shown to increase susceptibility to atherosclerosis [38]. The balance between mitochondrial, atherosclerosis-sensitizing and systemic, potentially atherosclerosis-protective, phenotypes of Mclk1+/− mice may explain the general absence of a significant effect on atherosclerosis that we observed. This balance may be modulated by genetic background, which could account for some of the contrasting results we observed between ApoE−/− mice of different backgrounds. Furthermore, Mclk1+/− mice also have higher levels of several pro-inflammatory cytokines (unpublished data). Inflammation plays a role in all stages of atherosclerosis.
[39], suggesting another possible reason why McIkl<sup>+/−</sup> mice are not protected against the disease.

**The effect of mutant clk-1 in C. elegans and McIk1 in mice on lipid metabolism**

*clk-1* mutant worms have altered oxidative and lipid metabolism [16,19]. The organismal differences between worms and mice, as well as other differences between the two models (homozygous mutants in worms and heterozygous mutants in mice) may explain why we did not see the expected effect on lipid metabolism and atherosclerosis in McIk1<sup>+/−</sup> mice. The *clk-1* mutant worms are homozygous for alleles incapable of producing UQ, resulting in an obvious, easily observable, phenotype [2,40]. On the other hand, mice retain one copy of the wild-type allele, and the MCLK1 produced appears suffi-
cient for a normal level of UQ synthesis [3,7,9]. It appears that, in both worms and mice, MCLK1/CLK-1 has an additional function besides the production of UQ. This is supported by evidence from both organisms. In worms, two mutant alleles of clk-1 yield phenotypes of different severity, despite the fact that neither allele can produce UQ [41,42]. Mclk1+/- mice actually have several very clear biochemical phenotypes despite wild-type levels of UQ [3,9]. Yet, the absence of a lipid phenotype in mice suggests the existence of a threshold that is not reached when CLK-1/MCLK1 activity is only reduced as in Mclk1+/- mouse mutants rather than completely abolished or very severely reduced as in the C. elegans mutants.

**Suppression of Mclk1+/- longevity by loss of ApoE**

In mice lacking the ApoE protein, Mclk1 heterozygosity no longer extends lifespan. In other words, some characteristic of the ApoE+/- background actually shortens the lifespan of Mclr1+/- mice relative to Mclkr1+/- mice. We also observed that Mclk1+/-; ApoE+/- mice weighed slightly less than Mclk1+/-; ApoE+/- mice, starting at approximately 1 year of age. This is in contrast to older Mclk1+/- mice wild-type for ApoE, which either weigh the same or more than wild-type controls [unpublished data and [1]]. This does imply that the absence of ApoE has detrimental effects on Mclk1+/- mice. An increased prevalence of atherosclerosis is unlikely to be the cause of this lifespan shortening effect. Firstly, as described above, Mclk1+/- mice do not appear more susceptible to atherosclerosis development. If anything, Mclk1+/- female mice in the aging experiment that were euthanized due to impending death showed a trend of slightly less aortic atherosclerosis than their wild-type controls. Secondly, it has been surprisingly difficult to find evidence in ApoE+/- mice of the arterial occlusion-induced cardiovascular events that are so lethal to humans [43]. Although older ApoE+/- mice may develop atherosclerosis of the coronary arteries, only a small proportion of these are found to display evidence of more advanced coronary artery disease, such as plaque rupture [24,44]. Aside from atherosclerosis, ApoE+/- mice suffer from other conditions, such as increased oxidative stress [45] and inflammation [46]. As described above, young Mclk1+/- mice have higher levels of oxidative stress in mitochondria as well as greater expression of certain cytokines consistent with a more pro-inflammatory state. Although these factors by themselves clearly do not prevent the increased longevity of Mclk1+/- mice, they may exert a negative effect that cancels out the lifespan extension when combined with corresponding phenotypes in ApoE+/- mice.

**Conclusion**

Contrary to our expectations based on the increased longevity of Mclk1+/- mice, Mclk1 heterozygosity does not protect against atherosclerosis. This is not surprising in light of recent findings that suggest a complex pattern of phenotypes due to Mclk1 heterozygosity, some of which may actually facilitate development of a disease such as atherosclerosis that involves oxidative stress and inflammation. Furthermore, Mclk1 heterozygosity does not extend lifespan in the ApoE-null background, suggesting that Mclk1 heterozygosity is not capable of protecting against the increased oxidative stress and inflammation that afflict ApoE-null mice.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

BH participated in the conception and design of this study, performed the experiments, participated in the analysis of data, and helped write the paper. SH participated in the conception and design of the study, participated in the analysis of data, and helped write the paper.

**Authors’ Information**

SH is Campbell Chair of Developmental Biology and Strathcona Chair of Zoology.

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**References**

1. Liu X, Jiang N, Hughes B, Bigras E, Shoubridge E, Hekimi S: Evolutionary conservation of the clk-1 dependent mechanism of longevity: loss of mclk1 increases cellular fitness and lifespan in mice. Genetics Dev 2005, 19(20):2397-2430.

2. Wong A, Boutis P, Hekimi S: Mutations in the clk-1 gene of Caenorhabditis elegans affect developmental and behavioral timing. Genetics 1995, 139(3):1247-1259.

3. Lapointe J, Stepanyan Z, Bigras E, Hekimi S: Reversal of the mitochondrial phenotype and slow development of oxidative biomarkers of aging in long-lived Mclk1+/- mice. J Biol Chem 2009 in press.

4. Ewbank JJ, Barnes TM, Lakowski B, Lussier M, Bushey H, Hekimi S: Structural and functional conservation of the Caenorhabditis elegans clk-1 gene. Science 1997, 275(5302):980-983.

5. Stenmark P, Grunler J, Mattsson J, Sindelar P, Nordlund P, Berthold DA: A new member of the family of di-iron carboxylate proteins. Coq7 (clk-1), a membrane-bound hydroxylase involved in ubiquinone biosynthesis. J Biol Chem 2001, 276(36):33297-33300.

6. Turunen M, Olsson J, Dallner G: Metabolism and function of coenzyme Q. Biochim Biophys Acta 2004, 1660(1-2):171-199.

7. Levavasseur F, Miyadera H, Sirois J, Tremblay ML, Kita K, Shoubridge E, Hekimi S: Ubiquinone is necessary for mouse embryonic development but is not essential for mitochondrial respiration. J Biol Chem 2001, 276(49):46160-46164.

8. Nakai D, Yusa S, Takahashi M, Shimitzu T, Asaumi S, Isono K, Takao T, Suzuki Y, Kuroyanagi H, Hirokawa K, Koseki H, Shirsawa T: Mouse homologue of coq7/clk, longevity gene in Caenorhabditis elegans, is essential for coenzyme Q synthesis, maintenance of mitochondrial integrity, and neurogenesis. Biochim Biophys Acta 2001, 1561(2-3):463-471.

9. Lapointe J, Hekimi S: Early Mitochondrial Dysfunction in Long-lived Mclk1+/- Mice. J Biol Chem 2008, 283(38):26217-26227.

10. Evert J, Lawler E, Bogan H, Perls T: Morbidity profiles of centenarians: survivors, delayers, and escapers. J Gerontol A Biol Sci Med Sci 2003, 58(3):232-237.
11. Andersen SL, Terry DF, Wilcox MA, Babineau T, Malek K, Perls TT: Cancer in the oldest old. Mech Ageing Dev 2005, 126(2):263-267.

12. Strong UK, Wrimen JL: Atherosclerosis. The Road Ahead. Cell 2001, 104(4):503-516.

13. Shibata Y, Branicky R, Landaverde IO, Hekimi S: Evolutionary conservation of drug action on lipoprotein metabolism-related targets. J Lipid Res 2007, 48(1):497-505.

14. Matyash V, McKinsey CA, Mankani M, Hensley A, Mukherjee S, Hirsh D, Thiele C, Grant B, Maxfield FR, Kurzchalia TV: Respiratory uncoupling in skeletal muscle delays death and diminishes age-related disease. Cell Metab 2007, 6(6):497-505.

15. Gates AC, Bernal-Mizrachi C, Chinault SL, Feng C, Schneider JG, Coleman T, Malone JP, Townsend RR, Chakravarthy MV, Semenov ICF: Recombinant age-related biologicals. Science 2003, 302(5651):1779-1782.

16. Napoli C, Martin-Padura I, de Nigris F, Giorgio M, Mansueto G, Calara F, Silvestre M, Casanada F, Yuan N, Napoli C, Palinski W: Redox regulation of germline and vulval development in Caenorhabditis elegans clk-1 mutants. Mol Biol Cell 2001, 12(6):1725-1736.

17. Smolenaars MM, Madsen O, Rodenburg KW, Horst DJ Van der: Molecu lar diversity and evolution of the large lipid transfer protein superfamily. J Lipid Res 2006, 48(3):489-502.

18. Hili A, Beauchamp MC, Brancicky R, Desjardins A, Casanova I, Guimon MP, Carroll R, Ethier M, Kianiana I, McBride K, Hekimi S: Evolutionary conservation of drug action on lipoprotein metabolism-related targets. J Lipid Res 2007, 49(1):74-83.

19. Bokov A, Chaudhuri A, Richardon A: The role of oxidative damage and stress in aging. Mech Ageing Dev 2004, 125(10-11):811-826.

20. Chang HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, Carter C, Yu BP, Leeuwenburgh C: Molecular inflammation: Underpinnings of aging and age-related diseases. Ageing Res Rev 2008, 8(1):18-30.

21. Zadelaar S, Kleemann R, Verschuren L, de Vries-Van der Weij J, van der Hoorn, Princen HM, Koosstra T, Mouse Models for Atherosclerosis and Pharmaceutical Modifiers. Arterioscler Thromb Vasc Biol 2007, 27(8):1706-1721.

22. Ohashi R, Mu H, Yao Q, Chen C: Cellular and molecular mechanisms of atherosclerosis with mouse models. Trends Cardiovasc Med 2006, 16(1):87-90.

23. Nakashima Y, Plump AS, Rains EW, Breslow JL, Ross R: ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb 1994, 14(1):133-140.

24. Strong JP, Malcom GT, McAlhan CA, Tracy RE, Newman WP, 3rd, Herderick EE, Cornell JL: Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA 1999, 281(8):727-735.

25. Higginson EA, Lambert G, McCarthy TC, Sinal GJ: Loss of functional farnesoid X receptor increases atherosclerotic lesions in apolipoprotein E-deficient mice. J Lipid Res 2005, 46(12):2595-2604.

26. Zhang Y, Wang X, Yales C, Lee FY, Lee H, Luisis AJ, Edwards PA: FXR deficiency causes reduced atherosclerosis in Ldlr-/- mice. Arterioscler Thromb Vasc Biol 2006, 26(10):2316-2321.

27. Merkel M, Velez-Carrasco W, Hughes LC, Breslow JL: Compared with saturated fatty acids, dietary monounsaturated fatty acids and carbohydrates increase atherosclerosis and VLDL cholesterol levels in LDL receptor-deficient, but not apolipoprotein E-deficient, mice. PNAS 2001, 98(23):13294-13299.

28. Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammar RE, Her J: Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenosivus-mediated gene delivery. J Clin Invest 1993, 92(2):883-893.

29. Piedrahita JA, Zhang SH, Hagaman JG, Oliver PM, Maeda N: Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci USA 1992, 89(10):4471-4475.