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TITLE: Effect of Ketone Bodies on Mitochondrial Cardiomyopathy and Heart Aging

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14. ABSTRACT
In this reporting period, we observed that fewer Tg-TMEM135 mice supplemented with ketone bodies in their diet died around the weaning age (postnatal day 21) compared to those that were on the regular diet. Additionally, we observed that Tg-TMEM135 mice on the control diet developed hypertrophy of the heart left ventricle by 21 days of age, whereas Tg-TMEM135 mice with ketone body supplementation did not. These results suggest that ketone body supplementation may be effective in improving/preventing the heart abnormalities caused by overexpression of TMEM135 in this mouse model. We have collected heart samples from these mice for histological and protein analyses, which will be performed in the next period. We are also in the process of aging out Tg-TMEM135 and WT control mice on ketone body supplementation to 8 months of age to test the long-term effect of this supplementation, as well as testing its effect of aging related changes of the heart in WT mice.

15. SUBJECT TERMS
Mitochondria, mitochondrial dynamics, mitochondrial disease, cardiomyopathy, mouse model, transmembrane protein 135 (TMEM135), aging, ketone body, supplementation

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1. Introduction

In this research, we use a novel mouse model of mitochondrial cardiomyopathy to test whether the heart disease can be prevented by supplementation of a ketone body, which is a metabolite of a specific amino acid whose metabolism is decreased in the heart of these mice. Since this mouse model shows similar heart conditions and gene expression profiles as aged mice, we further hypothesize that the ketone body supplementation may also prevent aging of the heart, which we will test in aging mice. If successful, we will lay a foundation for an innovative strategy to prevent mitochondrial cardiomyopathy and heart aging. Knowledge gained from this study can be applied to develop preventative approaches for heart symptoms in patients with mitochondrial diseases as well as for heart aging in the general population.

2. Keywords

Mitochondria, mitochondrial dynamics, mitochondrial disease, cardiomyopathy, mouse model, transmembrane protein 135 (TMEM135), aging, ketone body, supplementation

3. Accomplishments

-What were the major goals of the project?

(1) To test the effect of ketone bodies on the heart defects and sudden death of Tmem135 transgenic Tg-TMEM135) mice, a model for mitochondrial cardiomyopathy.

(2) To test the long-term effect of ketone bodies on the age-associated abnormalities of the heart

-What was accomplished under these goals?

(1) To test whether ketone bodies improve the heart conditions and survival rate of Tg-TMEM135 mice at weaning, we added ketone ester (KE), R,S-1,3-butanediol acetoacetate diester into the standard chow diet at 10% of volume. Saccharin was added at 1% for palatability. KE-diet was also supplied to wild-type (WT) mice. To Tg-TMEM135 and WT mice in the control group, the standard chow diet mixed with water was supplied. The KE or control treatment was carried out from postnatal day (P)15 (pups start eating solid food around this age) to examine the mortality around weaning on P21 (Fig. 1). We compared the survival curves of these four groups by Log-rank test and found that they are statistically different (P=0.0027).

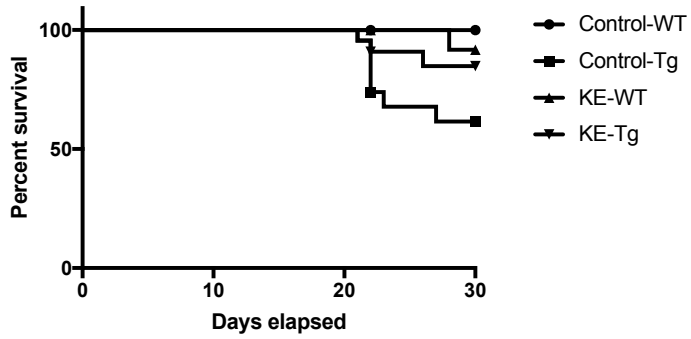

Fig. 1 Survival of Tg-TMEM135 and WT mice on KE or control diet around the weaning age.

Notably, eight out of 22 TMEM135-Tg mice that were fed control diet died around the weaning age, whereas 3 out of 22 TMEM135-Tg mice that were fed KE diet died around the weaning age although comparison of survival curves for these two groups alone does not reach statistical significance. We are in the process of increasing the number of animals for this analysis.

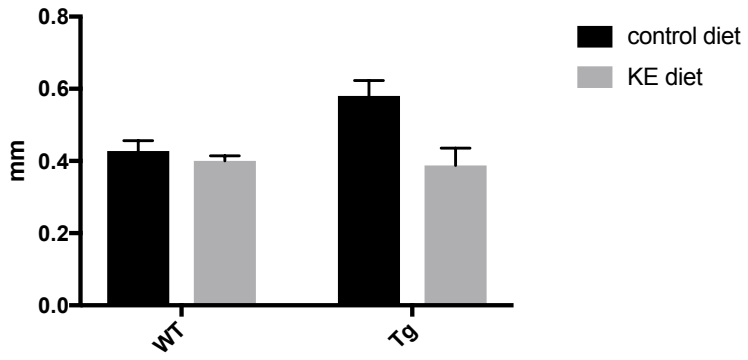

Fig. 2 Left ventricle (LV) posterior wall thickness at end-diastole of WT and Tg-TMEM135 mice on control or KE diet at P21.

We measured the heart functions of KE-treated and non-treated TMEM135-Tg mice as well as KE-treated and non-treated wild-type control mice by echocardiograms at P21. We observed that the left ventricle (LV) posterior and anterior wall thickness at end-diastole are significantly increased in TMEM135-Tg mice on the control diet compared to those of WT control mice as well as those in KE-treated TMEM135-Tg and WT control mice (Fig. 2; p=0.0001, 2-way ANOVA with Bonferroni's multiple comparison test). These results show that the left ventricle wall thickness is increased in TMEM135-Tg mice compared to WT mice by P21 on control diet, while this increase is not observed in TMEM135-Tg mice that were fed KE-diet (P15-P21), suggesting that KE diet may prevent the development of LV hypertrophy in TMEM135-Tg mice. We have collected heart samples of mice from these groups at P22 for histological analysis and protein (western blot) analysis.

In order to test the long-term effect of ketone bodies on the Tg-TMEM135 mouse heart, we are feeding KE diet to 13 Tg-TMEM135 mice along with 11 wild-type control mice, and control diet to 9 Tg-TMEM135 mice and 11 control mice from P15 to 8 months. Echocardiograms were recorded from some of these mice and the results are currently being analyzed. We have also collected heart tissues from these mice for histological analysis as well as protein analysis. We will age the rest of the mice to 8 months of age, and perform echocardiography and collect tissues for histological/protein analyses at that time.

(2) In order to test the effect of ketone bodies on pathological changes of the heart associated with aging, we have added KE to the chow diet of C57BL/6J mice (n=10) from P15. These mice, along with the control group on non-treated control diet (n=10), will be aged out to 15 months, at which time we will measure the heart function by echocardiography and collect samples for histological and protein analysis of their hearts.

-What opportunities for training and professional development has the project provided?
The project has provided opportunities for Assistant Scientist, Wei-hua Lee to be trained to develop as an independent researcher. She is learning how to manage and carry out the project.

-How were the results disseminated to communities of interest?
Nothing to report

-What do you plan to do during the next reporting period to accomplish the goals?
(1) We will increase the number of mice for analyzing the survival rate around the weaning age. We will perform histological analysis and protein analysis of the heart of Tg-TMEM135 and WT mice that were on KE or control diet from P15 to P22. For the long-term effect, we will complete analysis of echocardiography and perform histological and protein analysis of the heart of Tg-TMEM135 and WT mice that were on KE or control diet from P15 to 8 months.

(2) We will continue feeding KE or control diet to C57BL/6J mice until 15 months of age. We will then analyze the heart function by echocardiography and collect heart samples for histological and protein analysis. We will perform these analyses and test whether ketone body supplementation improves aging-related changes of the heart.

4. Impact

-What was the impact on the development of the principal discipline of the project?
The results obtained in this reporting period indicate that ketone body supplementation improved the survival of Tg-TMEM135 mice, a mouse model of mitochondrial cardiomyopathy, around the weaning age. It also prevented left ventricle hypertrophy of the heart observed in Tg-TMEM135 mice at this age. While further analysis of heart samples obtained during this period is still underway, these results suggest the possibility that ketone body supplementation may be effective in improving/preventing mitochondrial cardiomyopathy.

-What was the impact on other disciplines?
Nothing to report

-What was the impact on technology transfer?
Nothing to report

-What was the impact on society beyond science and technology?
Nothing to report

5. Changes/Problems

-Changes in approach and reasons for change
Nothing to report

-Actual or anticipated problems or delays and actions or plans to resolve them
Since our institutional animal protocol that covers the work for this award was up for renewal and renewal was approved by IACUC on 11/16/17, we needed to submit the documents to ACURO for review (11/29/17). The renewed protocol was approved by ACURO on 2/7/18. This has delayed the project by approximately 2 months.

-Changes that had a significant impact on expeditures
Nothing to report

-Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

6. Products, Inventions, Patent Applications, and/or Licenses
Nothing to report.

7. Participants & Other Collaborating Organizations
-What individuals have worked on the project?
Name: Akihiro Ikeda, Ph.D., D.V.M.
Project Role: PI
Researcher Identifier: ORCID ID: http://orcid.org/0000-0001-8440-3891
Nearest person months worked: 1.8
Contribution to Project: Dr. Ikeda has been responsible for the experimental design, carrying out the experiments outlined, interpretation of data, supervision and coordination of the project.

Name: Wei-hua Lee, Ph.D.
Project Role: Assistant Scientist
Nearest person months worked: 6
Contribution to Project: Dr. Lee has been responsible for carrying out the molecular biological experiments outlined, managing the mouse colony, phenotyping of mice and interpretation of data under the supervision of Dr. Ikeda.

-Has there been a change in the active other support of the PD/PI or senior / key personnel since the last reporting period?
Akihiro Ikeda (PI) has acquired the following funding during the reporting period:

Title: Role of chondroitin sulfate proteoglycans in photoreceptor - RPE interaction (R21 EY029067)
Time commitment: 1.2 calendar months
Supporting Agency: NIH/NEI
Period: 4/1/18-3/30/20
Major goal: To understand the roles chondroitin sulfate synthese 1 plays in the retina, and how a defect in this molecule causes accelerated aging phenotypes.
Role: PI
Overlap: None

-What other organizations were involved as partners?
Nothing to report.

8. Special Reporting requirements
None

9. Appendices
None