60th Medical Group (AMC), Travis AFB, CA

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

FINAL REPORT SUMMARY

(Please type all information. Use additional pages if necessary.)

PROTOCOL #: FDG20150003A **DATE:** 7 April 2016

PROTOCOL TITLE: : "Effect of Diet High in Coconut Oil on Cardiovascular Disease Risk in ApoE Knockout and Wild Type Mice (*Mus musculus*)."

PRINCIPAL INVESTIGATOR (PI) / TRAINING COORDINATOR (TC): Capt Jeffrey Perry

DEPARTMENT: Nutritional Medicine **PHONE #:** 707-423-2374

INITIAL APPROVAL DATE: 24 November 2014 **LAST TRIENNIAL REVISION DATE:** 19 November 2015

FUNDING SOURCE: AF Surgeon General

1. **RECORD OF ANIMAL USAGE:**

Animal Species:	Total # Approved	# Used this FY	Total # Used to Date
Mus musculus	**50**	**50**	**50**

2. **PROTOCOL TYPE / CHARACTERISTICS:** (Check all applicable terms in **EACH** column)

___ Training: Live Animal	___ Medical Readiness	___ Prolonged Restraint
___ Training: non-Live Animal	___ Health Promotion	___ Multiple Survival Surgery
_X_ Research: Survival (chronic)	_X__ Prevention	___ Behavioral Study
___ Research: non-Survival (acute)	___ Utilization Mgt.	___ Adjuvant Use
___ Other ( )	___ Other (Treatment)	___ Biohazard

3. **PROTOCOL PAIN CATEGORY (USDA):** (Check applicable) ___ C _X_ D ___ E

4. **PROTOCOL STATUS:**

***Request Protocol Closure:**

___ Inactive, protocol never initiated

___ Inactive, protocol initiated but has not/will not be completed

_X__ Completed, all approved procedures/animal uses have been completed

5. **Previous Amendments:**
List all amendments made to the protocol.. **IF none occurred, state NONE. Do not use N/A.**

For the Entire Study Chronologically

Amendment Number	Date of Approval	Summary of the Change
None		

6. **FUNDING STATUS:** Funding allocated: $9470.00 Funds remaining: $ 0

7. **PROTOCOL PERSONNEL CHANGES:**

Have there been any personnel/staffing changes (PI/CI/AI/TC/Instructor) since the last IACUC approval of protocol, or annual review? ___ Yes _X__ No

If yes, complete the following sections (Additions/Deletions). For additions, indicate whether or not the IACUC has approved this addition.

ADDITIONS: (Include Name, Protocol function - PI/CI/AI/TC/Instructor, IACUC approval - Yes/No)

DELETIONS: (Include Name, Protocol function - PI/CI/AI/TC/Instructor, Effective date of deletion)

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8. **PROBLEMS / ADVERSE EVENTS:** Identify any problems or adverse events that have affected study progress. Itemize adverse events that have led to unanticipated animal illness, distress, injury, or death; and indicate whether or not these events were reported to the IACUC.

The timeline for this study was extended due to the PI being sent TDY for six weeks. This was reported to the IACUC as it did not affect the animals in any way other than them living for a longer period of time.

9. **REDUCTION, REFINEMENT, OR REPLACEMENT OF ANIMAL USE:**

REPLACEMENT (ALTERNATIVES): Since the last IACUC approval, have alternatives to animal use become available that could be substituted in this protocol without adversely affecting study or training objectives?

No.

REFINEMENT: Since the last IACUC approval, have any study refinements been implemented to reduce the degree of pain or distress experienced by study animals, or have animals of lower phylogenetic status or sentience been identified as potential study/training models in this protocol?

No.

REDUCTION: Since the last IACUC approval, have any methods been identified to reduce the number of live animals used in this protocol?

No.

10. **PUBLICATIONS / PRESENTATIONS:** (List any scientific publications and/or presentations that have resulted from this protocol. Include pending/scheduled publications or presentations).

A manuscript is in preparation.

11. **Were the protocol objectives met, and how will the outcome or training benefit the DoD/USAF?**

Yes. Although the results were negative, the protocol provided a valuable training opportunity for a BSC officer.

12. **PROTOCOL OUTCOME SUMMARY:** (Please provide, in "ABSTRACT" format, a summary of the protocol objectives, materials and methods, results - include tables/figures, and conclusions/applications.)

Objective: The goal of this study was to evaluate the risk of cardiovascular disease in both a control (B6/C57J) and a proatherosclerotic (ApoE -/-) mouse model when consuming diets high in coconut oil compared to a high-fat control.

Methods: Female control B6/C57J and ApoE -/- knockout mice were obtained from JAX Labs and acclimated to the facility. The mice were weighed and randomly assigned to receive a custom diet with either coconut oil or milk fat. Both diets were formulated to have the same amount of protein, carbohydrates, and fat and were provided *ad libitum*. The mice and the food were weighed weekly. After 14 weeks, the mice were sacrificed with CO_2, and

blood, aorta, and liver samples were obtained. Blood samples were pooled by cage and analyzed to determine triglycerides, HDL/LDL cholesterol, and total cholesterol. Aorta and liver specimens were routinely processed and evaluated by a pathologist blinded to treatment.

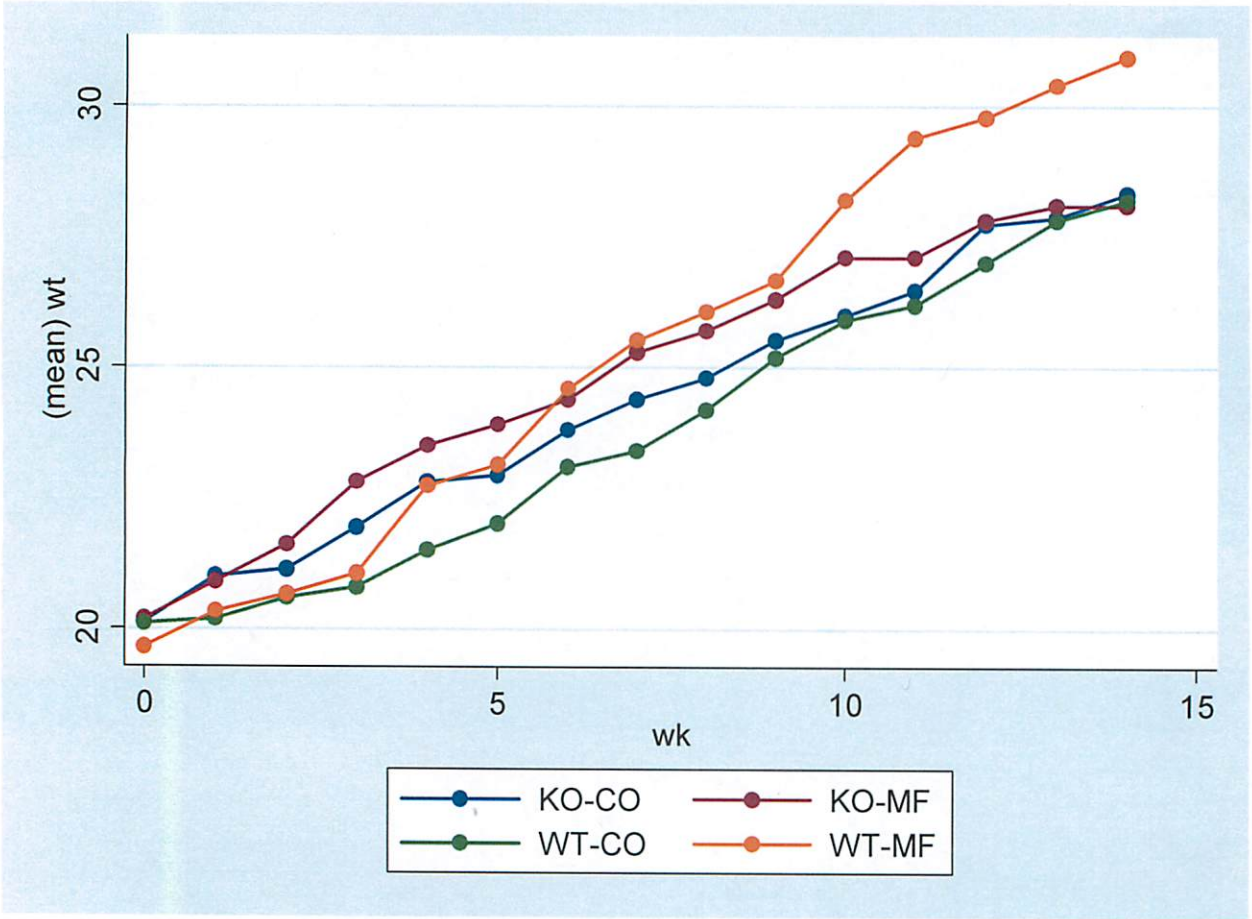
Results: As seen in the following tables, there were no differences in the average (by cage) weight gain or amount of diet consumed regardless of genotype or diet consumed. Similarly, there were no differences in total cholesterol, HDL, and triglyceride in any of the groups. The pathology results were more revealing, with statistically significant differences between knockout and wildtype mice in aorta score regardless of diet, and in liver score with coconut oil diet. Statistically significant differences by diet were seen in aorta score for knockout mice and in liver score for wildtype mice, but scores were higher for mice consuming milk fat than for those consuming coconut oil.

Average Total Diet Consumed (g)

Mouse Type	Diet		P-value
	Coconut Oil	Milk Fat	
Knockout	292.9 ± 28.4	294.2 ± 42.0	1.00
Wildtype	293.1 ± 25.5	294.0 ± 45.4	0.56
P-value	0.56	0.56	

Average Weight Gain (g)

Mouse Type	Diet		P-value
	Coconut Oil	Milk Fat	
Knockout	8.2 ± 0.8	7.9 ± 4.6	1.00
Wildtype	8.1 ± 1.0	12.4 ± 4.6	0.25
P-value	0.77	0.25	



Total Cholesterol (mg/dL)

Mouse Type	Diet		P-value
	Coconut Oil	Milk Fat	
Knockout	2068.2 ± 172.8	1965.7 ± 47.2	0.25
Wildtype	164.9 ± 22.1	168.1 ± 46.1	0.56
P-value	0.08	0.08	

HDL (mg/dL)

Mouse Type	Diet		P-value
	Coconut Oil	Milk Fat	
Knockout	17.8 ± 7.1	20.7 ± 12.6	1.00
Wildtype	154.7 ± 19.2	159.6 ± 45.1	1.00
P-value	0.08	0.08	

Triglyceride (nM/µL)

Mouse Type	Diet		P-value
	Coconut Oil	Milk Fat	
Knockout	1.25 ± 1.1	1.49 ± 0.6	1.00
Wildtype	1.0 ± 0.7	0.7 ± 0.3	0.25
P-value	0.56	0.08	

Aorta Scoring:
0 = No pathology
1 = Small plaque
2 = Focal lesion
3 = Multifocal lesions

Aorta Score

Mouse Type	Diet		P-value
	Coconut Oil	Milk Fat	
Knockout	2 ± 2	3 ± 1	0.03
Wildtype	0 ± 0	0 ± 0	
P-value	<0.01	<0.01	

Liver Scoring:
0 = No pathology
1 = Periportal lipid vacuoles
2 = Midzonal lipid vacuoles
3 = Centrilobular lipid vacuoles

Liver Score

Mouse Type	Diet		P-value
	Coconut Oil	Milk Fat	
Knockout	1 ± 1	1 ± 1	0.67
Wildtype	0 ± 1	1 ± 0	0.02
P-value	<0.01	0.12	

Conclusions: The mice used in this study had normal dietary consumption and weight gain, regardless of diet. This is not too surprising, given that both diets had the same nutritional and caloric contents. The differences in aorta and liver scores between knockout and wildtype mice are readily explained by the absence of the ApoE gene in the knockout mice. ApoE is an anti-atherosclerotic protein made by the liver and incorporated into circulating lipoproteins. When ApoE is absent, proatherosclerotic lipoproteins accumulate in the blood promoting the formation of atherosclerotic plaques on blood vessels. Milk fat consumption resulted in significant increases in aorta scores among knockout mice and liver scores in wildtype mice. These results were unexpected.

(PI / TC Signature)

14 April 2016
(Date)

Attachments:
Attachment 1: Defense Technical Information Center (DTIC) Abstract Submission **(Mandatory)**

Attachment 1
Defense Technical Information Center (DTIC) Abstract Submission

Objective: We evaluated the risk of cardiovascular disease in both control and proatherosclerotic mice consuming diets high in coconut oil.

Methods: The mice were weighed and randomly assigned to receive a custom diet with either coconut oil or milk fat. Both diets were formulated to have the same amount of protein, carbohydrates, and fat and were provided *ad libitum*. The mice and the food were weighed weekly. Blood samples were pooled by cage and analyzed to determine triglycerides, HDL/LDL cholesterol, and total cholesterol. Aorta and liver specimens were routinely processed and evaluated by a pathologist blinded to treatment.

Results: There were no differences in the average weight gain or amount of diet consumed regardless of genotype or diet consumed. Similarly, there were no differences in total cholesterol, HDL, and triglyceride in any of the groups. Statistically significant differences were seen between knockout and wildtype mice in aorta score regardless of diet, and in liver score with coconut oil diet. Statistically significant differences by diet were seen in aorta score for knockout mice and in liver score for wildtype mice, but scores were higher for mice consuming milk fat than for those consuming coconut oil.

Conclusion: Milk fat consumption resulted in significant increases in aorta scores among knockout mice and liver scores in wildtype mice. These results were unexpected. However, the clinical significance of the increased scores is unknown.

Grant Number:_____
From:_____
****If you utilized an external grant, please provide Grant # and where the grant came from. Thank you.**