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Award Number: DAMD17-03-1-0258

TITLE: Effect of Dietary Intervention on Prostate Tumor Development in TRAMP Mice

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REPORT DATE: May 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 01-05-2006		2. REPORT TYPE Annual		3. DATES COVERED 1 May 2005 –30 Apr 2006	
4. TITLE AND SUBTITLE Effect of Dietary Intervention on Prostate Tumor Development in TRAMP Mice				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER DAMD17-03-1-0258	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Margot P. Cleary				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Minnesota Austin, MN 55912				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES Original contains colored plates: ALL DTIC reproductions will be in black and white.					
14. ABSTRACT Calorie restriction has been reported to protect rodents from many different cancers. With respect to prostate cancer, a protective effect of energy restriction on development of spontaneous prostate tumors in Lobund-Wistar rats and tumors developing from transplanted prostate tumor tissue or cells in mice and rats have been published. However we have found that in female rodents intermittent caloric restriction is more protective than chronic restriction in preventing transgenic mammary tumor development. Here, we determined how intermittent versus chronic calorie restricted affected development of prostate cancer in transgenic TRAMP mice. A 25% reduction in caloric intake is being utilized. Initial findings indicate that intermittent-restricted mice have a significant delay in the age of tumor detection and in the age of death. Evaluation of histopathology is underway and we are attempting to identify a metabolic pathway to target for prevention and/ treatment strategies. In particular we are assessing aspects of the IGF-I and leptin axes. The results of this study provide further evidence that the manner in which calories are consumed has a significant impact of development of some malignancies.					
15. SUBJECT TERMS Prostate cancer, TRAMP, transgenic mice, caloric restriction					
16. SECURITY CLASSIFICATION OF:			UU	18. NUMBER OF PAGES 21	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION:

A number of prospective epidemiological studies indicate that as body weight and/or energy intake increase so does the risk for prostate cancer. In rodent studies chronic calorie restriction is associated with extended life expectancy and decreased incidence of many malignancies. Due to a lack of suitable animal models of prostate cancer, only a few studies have addressed issues of nutrition intervention in the progression of this disease. However, results of these studies support a protective effect of energy restriction on spontaneous prostate tumor development in Lobund-Wistar rats [1;2] and on transplanted tumor/cell prostate tumor growth in mice and rats [3], although a mechanism of action has not been identified. There are limitations to the application of these models to the human disease process. Recent introduction of the TRAMP (transgenic adenocarcinoma mouse prostate) mouse provides a model that shares many characteristics with human prostate cancer [4;5], but their use in nutritional studies has been limited. We are using TRAMP mice to evaluate their response to chronic calorie restriction, as well as to intermittent caloric restriction/refeeding. These studies are based on our recent report that these two interventions resulted in decreased incidence and extended latency of oncogene-induced mammary tumors in MMTV-TGF- α female mice [6]. Furthermore, we found that the intermittent caloric restriction/refeeding regimen was more protective than chronic restriction. In the present study TRAMP mice have been followed to determine their response to these interventions with respect to age of prostate cancer detection and metastases rates. Serum and tissue samples have been obtained to determine the role of the insulin-like growth factor (IGF) axis in the protective action of caloric restriction. We have also begun tissue analyses for other pathways that may be involved such as apoptosis and leptin action.

BODY:

Progress in relation to Revised Statement of Work 2/13/03 (attached)

TASK 1 & 2. Establish breeding colony & set up genotyping assay.

MONTH 0-3. Order 6 male TRAMP mice (maximum number that could be ordered at one time) and 25 nontransgenic female mice for breeding. Set up breeding. Set up genotyping assay and genotype mice produced. Rebreed mice to expand breeding colony.

Progress: Now completed with respect to breeding regimens and genotyping.

TASK 3. Breed mice for EXPERIMENT 1A- LONGITUDINAL STUDY.

MONTHS 4-6. Breed mice to produce one third to one half of mice needed for this study. If one estimates 8 pups per litter, 1 out of 4 pups will be TRAMP males = two TRAMP males per litter. We will need a total of 160 TRAMP males = 80 litters. Genotype offspring. Assign mice to experimental groups. Set up immunohistochemistry assays.

Progress: Breeding completed.

TASK 4. Complete enrollment of mice for EXPERIMENT 1A and 1B SERIAL STUDY.

MONTHS 7-12. Continue breeding to compete EXPERIMENT 1A. Three to four rounds of breeding will probably be needed to supply enough mice. Genotype mice as they are produced. Assign mice to experimental groups. Once longitudinal study is complete begin assigning mice to serial study for EXPERIMENT 1B.

Progress: Breeding completed.

TASK 5. Follow mice in EXPERIMENT 1A and 1B.

MONTHS 6-21. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When age, tumor size and/or animal condition dictates euthanize mice and perform autopsies. Euthanize nontransgenic age-matched mice to correspond to those TRAMP mice with tumors. Euthanize mice that reach terminal ages of 48 or 50 wk of age. Record results and when study complete do statistical analyses of results.

Progress: All mice have been euthanized. We are in the process of completing compiling the pathology reports to determine tumor and metastases status. A large amount of data has been obtained and is in the process of being summarized and prepared for statistical analyses and to determine what samples to analyze further. Here we will highlight what we have obtained to date.

Food Intake. Food intakes for the mice during each of the four week cycles of the experiment are shown in Table 1. By prevention of overeating during the refeeding period we maintained an overall degree of restriction of ~25% for the intermittent-restricted and chronic-restricted (pair-fed) groups. This will make interpretation of the results more straight forward as we found previously with female mice that some of them overate relative to the ad libitum fed mice during the refeeding stages resulting in overall caloric restriction being in the range of 10-20% although still highly protective [7] (Cleary et al manuscript submitted).

Table 1. Average Daily Food Intake (mean grams/day $\pm$ sd) for TRAMP Mice*

	Ad libitum-fed	Intermittent-Restricted	Pair-fed
Cycle 1	5.63 $\pm$ 0.79 (n=140)	4.2 (n=216)	4.2 (n=184)
Cycle 2	5.33 $\pm$ 0.69 (n=140)	4.0 (n=213)	4.0 (n=183)
Cycle 3	5.12 $\pm$ 0.46 (n=118)	3.8 (n=196)	3.8 (n=156)
Cycle 4	4.91 $\pm$ 0.71 (n=99)	3.7 (n=185)	3.7 (n=147)
Cycle 5	4.65 $\pm$ 0.72 (n=90)	3.5 (n=177)	3.5 (n=137)
Cycle 6	4.46 $\pm$ 0.70 (n=74)	3.3 (n=149)	3.3 (n=111)
Cycle 7	4.41 $\pm$ 0.51 (n=58)	3.3 (n=127)	3.3 (n=89)
Cycle 8	4.29 $\pm$ 0.41 (n=49)	3.2 (n=118)	3.2 (n=83)
Cycle 9	4.25 $\pm$ 0.50 (n=38)	3.2 (n=103)	3.2 (n=68)
Cycle 10	4.19 $\pm$ 0.39 (n=22)	3.1 (n=85)	3.1 (n=49)
Cycle 11	4.13 $\pm$ 0.76 (n=16)	3.1 (n=42)	3.1 (n=42)

*Includes mice from both longitudinal and cross sectional studies.

PROGRESS LONGITUDINAL STUDY

Body weight. The body weight curves for the mice in the Longitudinal Study are shown in Figure 1. In contrast to our earlier study using female TGF- α mice the intermittent restricted mice did not regain weight to reach the body weight attained for the ad libitum-fed mice during refeeding periods. However, as indicated above in this protocol we are restraining the food intake of the mice during the refeeding periods so that they do not “overshoot” the intake of the ad libitum fed mice. The ad libitum fed mice exhibited a slow steady increase in body weight until about 40 weeks of age and then a plateau was reached. The chronic restricted mice had a moderate weight gain at the beginning of the intervention and then their body weights were maintained. As expected from the experimental design the intermittent restricted mice lost weight during the restriction phases and regained body weight with refeeding. Note that the last body weight recorded on the body weight graph represents the one week of refeeding as mice were euthanized during the 50th week.

BODY WEIGHT OF TRAMP MICE OVER THE COURSE OF THE LONGITUDINAL STUDY

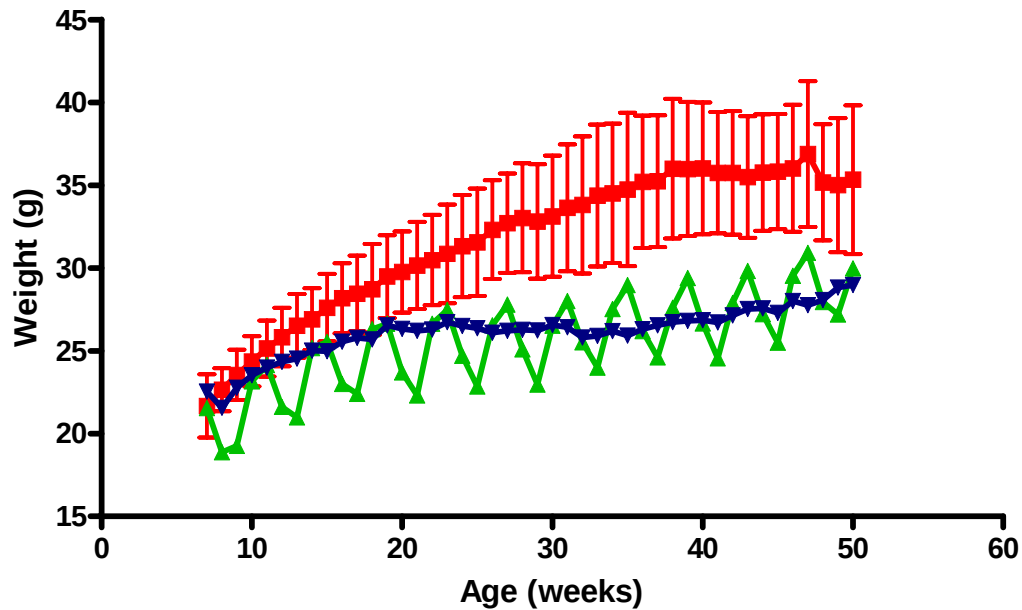


Figure 1: Body Weight curves for TRAMP mice in longitudinal study. Ad libitum (■) n=4-41 depending upon age; Intermittent-Restricted (▲), n=22-101 dependent upon age; Chronic-Restricted (▼) n=21-79 dependent upon age. For clarity error bars not included for the two restricted groups. ANOVA $p < 0.0001$, Ad libitum groups significantly different from each restricted group a $p < 0.001$ while there was no difference between the two restricted groups.

Final body weights for the mice are shown in Figure 2. Here the intermittent restricted mice are separated for those euthanized (or designated to be euthanized) at 48 and 50 weeks of age. It can be seen that the ad libitum mice weigh more than all other groups while the intermittent restricted mice euthanized at 50 weeks of age had significantly higher body weights than both the intermittent restricted-48 and the chronic restricted groups.

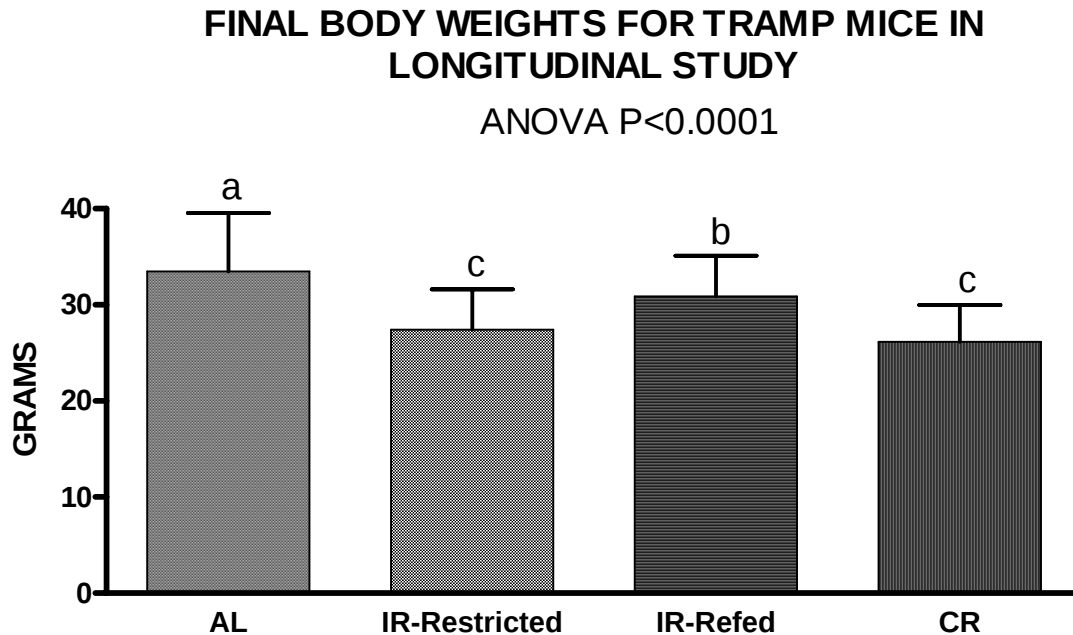


Figure 2. Final body weights for TRAMP mice in Longitudinal Study. AL = Ad libitum-fed mice (n = 39); IR-Restricted= Intermittent-Restricted to be euthanized at 48 weeks of age after final restriction (n = 41); IR-Refed =Intermittent-Restricted to be euthanized at 50 weeks of age after final refeeding cycle (n = 55); CR = Chronic-Restricted (n = 75). ANOVA $p < 0.0001$; columns with different superscripts significantly different from each other.

Additional information for the mice is shown in Figure 3 indicating that more mice in the intermittent group, a 300% increase, lived to the terminal end point of the experiment (48 or 50 weeks of age) compared to ad libitum fed mice. The chronic group had an intermediate response. These data are presently being analyzed by the statistician.

PERCENT OF TRAMP MICE LIVING TO TERMINAL AGE IN INTERMITTENT RESTRICTION STUDY

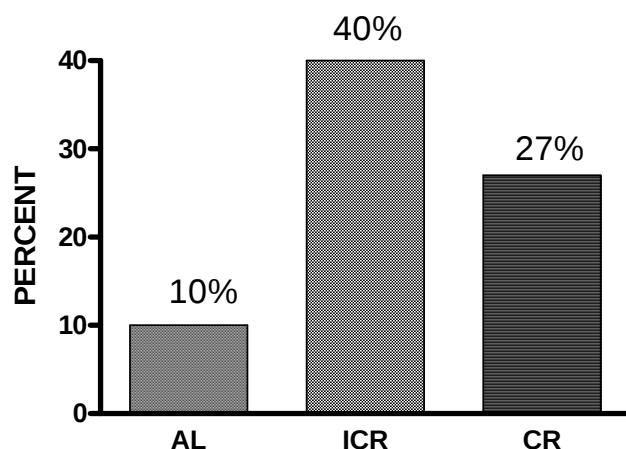


Figure 3: Bar graph representation of percent of mice in experimental groups living to terminal ages. Ad libitum fed (AL), Intermittent restricted (ICR) and Chronic restricted (CR). These results are presently being analyzed by statistician.

Additionally the data for age of tumor detection (Figure 4) and age at death (Figure 5) are presented in Kaplan Meier Plots with Chi Square Analysis. In both cases the intermittent restricted mice exhibited shifts to the right indicating significantly older age at tumor detection and death.

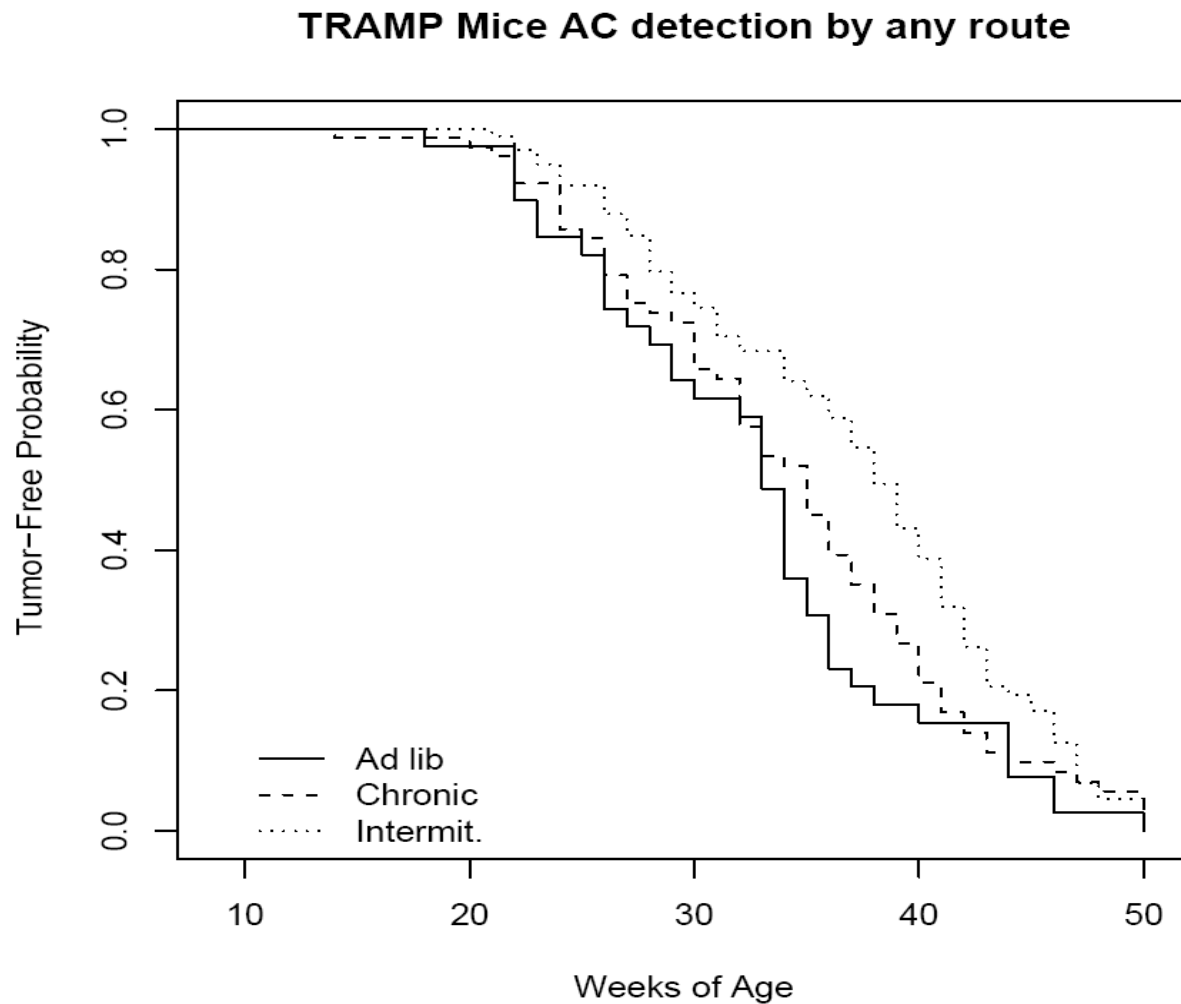


Figure 4: Age of tumor detection as exhibited by K-M plot. The curve comparison for ad libitum-fed and chronic restricted mice is not significant. Intermittent restricted mice had significantly delayed age of tumor detection compared to ad libitum ($p < 0.005$) mice. Furthermore, the difference between intermittent and chronic restricted mice just missed statistical significance ($p = 0.056$).

TRAMP Mice All Cause Mortality

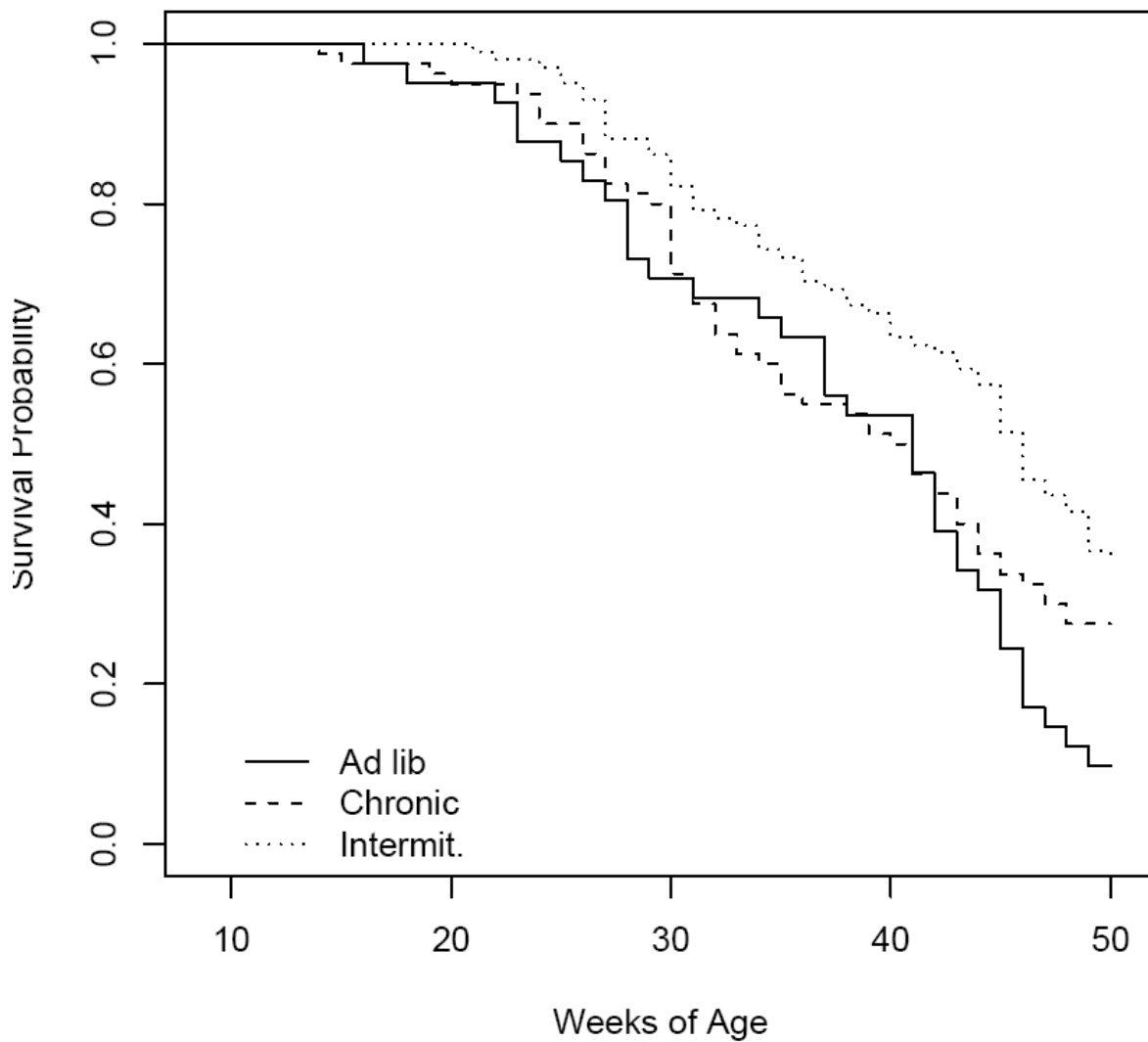


Figure 5. Kaplan-Meier survival curve for TRAMP mice in the Intermittent Restriction Study. There was no significant difference between the ad libitum fed and chronic restricted groups. However, intermittent restricted mice lived longer in comparison to both ad libitum ($p < 0.0005$) and chronic restricted ($p < 0.0476$) mice.

We have recently obtained data on body composition from NCI. The results are presented in Table 2. Some wild-type mice designated as nonTRAMP corresponding to each diet group are included for comparison. These data have not been analyzed by the statistician yet. However, it primarily looks like bone growth was similar in all groups and body fat was reduced in the two restricted groups. At this time all the intermittent mice are included as one group.

Table 2. Body Composition Results for TRAMP Mice in the Longitudinal Study.

Dietary Group	N	Bone Data			Tissue Data			
		BMD (g/cm ²)	BMC (g)	AREA (g/cm ²)	Lean (g)	Fat (g)	Total (g)	% Fat
AL TRAMP	24	0.05 ± 0.00	0.58 ± 0.09	10.97 ± 1.00	12.47 ± 2.56	6.68 ± 2.72	19.15 ± 4.73	34.05 ± 7.34
AL nonTRAMP	9	0.05 ± 0.00	0.64 ± 0.10	11.74 ± 1.06	14.16 ± 2.05	11.70 ± 4.25	25.88 ± 5.31	44.23 ± 8.30
IR TRAMP	70	0.05 ± 0.00	0.55 ± 0.08	10.55 ± 0.92	12.86 ± 3.16	5.19 ± 1.84	18.04 ± 3.96	28.78 ± 7.91
IR nonTRAMP	23	0.05 ± 0.00	0.52 ± 0.05	10.34 ± 0.84	13.96 ± 2.44	4.84 ± 1.50	18.78 ± 2.66	25.85 ± 7.13
CR TRAMP	52	0.05 ± 0.00	0.53 ± 0.07	10.38 ± 0.87	14.23 ± 3.06	4.84 ± 1.78	19.08 ± 3.99	25.32 ± 7.20
CR nonTRAMP	20	0.05 ± 0.00	0.57 ± 0.06	10.35 ± 0.74	16.61 ± 2.50	6.87 ± 3.06	23.47 ± 3.69	28.62 ± 9.30

AL = Ad libitum, IR = Intermittent-Restricted, CR = Chronic-Restricted.
Statistics not completed yet

A summary of some of the results are presented in Table 3. In general it can be seen that with respect to age of tumor detection, age at death and survival until study termination are all improved to a greater degree in the intermittent restricted mice compared to the chronic restricted mice. This is highlighted by the direct comparison of the two restricted groups receiving the same caloric and nutrient intakes with only the manner of consumption whereby the intermittent restricted mice have a delay in tumor detection and a delay in death.

Table 3. Highlights of Comparisons Among TRAMP Mouse Groups in Longitudinal Study.

	Ad libitum vs Intermittent-Restricted	Ad libitum vs Chronic-Restricted	Intermittent-Restricted vs Chronic-Restricted
Food intake	25% ↑	25% ↑	same
Tumor Number	same	same	same
Tumor Weight	same	same	same
Age at Tumor Palpation	14 % ↓	7 % ↓	7 % ↑
Age at Death	10 % ↓	3 % ↓	7 % ↑
Survival until study termination	75 % ↓	63 % ↓	48 % ↑

PROGRESS CROSS-SECTIONAL STUDY

In the Cross-Sectional Study mice were euthanized at predetermined ages for tissue collection. This included 7, 16 & 18, 28 & 30, and 40 & 42 weeks of age. The primary goal was to collect tissue samples at various stages of disease development. Summaries of the final body weights and genital-urinary tract weights are shown in Tables 4-6 for ages 16 & 18, 28 & 30, and 40 & 42, respectively. With the completion of pathology analyses we have begun analyses of the tissues available. We are also planning to use tissue blocks for immunohistochemistry. Proteins of interest included IGF-Receptor, Leptin receptor, apoptosis related proteins including Bcl-2, caspase-3 and Bax.

Table 4: Body and Genital-Urinary Tract Weight in TRAMP Mice at 16 and 18 Weeks of Age (mean $\pm$ s.d.)

	AL 16 N=9	IR 16 N=8	CR 16 N=7
Final BW	27.6 $\pm$ 2.0 ^a	22.4 $\pm$ 2.0 ^b	26.7 $\pm$ 1.3 ^a
GU weight	1.1 $\pm$ 0.2 ^a	0.7 $\pm$ 0.2 ^b	1.0 $\pm$ 0.1 ^a
Tumor detection rate	No tumors	No tumors	No tumors
	AL 18 N=8	IR 18 N=8	CR 18 N=8
Final BW	29.1 $\pm$ 2.5 ^a	25.7 $\pm$ 1.7 ^b	28.7 $\pm$ 1.8 ^A
GU weight	1.1 $\pm$ 0.2 ^a	0.74 $\pm$ 0.3 ^b	1.3 $\pm$ 0.3 ^a
Tumor detection rate	No tumors	No tumors	1 tumor found at dissection

Across a row mice with different superscript letters are significantly different.

Table 5: Body and Genital-Urinary Tract Weight in TRAMP Mice at 28 and 30 Weeks of Age (mean $\pm$ s.d.)

	AL 28 N=5	IR 28 N=7	CR 28 N=10
Final BW	30.2 $\pm$ 1.0 ^a	30.4 $\pm$ 2.8 ^a	32.1 $\pm$ 3.1 ^a
GU weight	3.67 $\pm$ 2.1 ^a	1.7 $\pm$ 0.4 ^b	1.8 $\pm$ 0.5 ^b
Tumor detection rate	3	2	3
	AL 30 N=5	IR 30 N=9	CR 30 N=6
Final BW	33.7 $\pm$ 2.1 ^a	32.0 $\pm$ 3.3 ^a	31.6 $\pm$ 3.1 ^a
GU weight	3.8 $\pm$ 2.8 ^a	3.2 $\pm$ 2.7 ^a	3.0 $\pm$ 3.2 ^a
Tumor detection rate	3	5	1

Across a row mice with different superscript letters are significantly different.

Table 6: Body and Genital-Urinary Tract Weight in TRAMP Mice at 40 and 42 Weeks of Age (mean $\pm$ s.d.)

	AL 40 N=3	IR 40 N=3	CR 40 N=5
Final BW	34.1 $\pm$ 3.0 ^a	28.3 $\pm$ 1.8 ^a	33.5 $\pm$ 2.9 ^a
GU weight	5.3 $\pm$ 0.3 ^a	2.8 $\pm$ 0.5 ^b	4.3 $\pm$ 0.6 ^a
Tumor detection	3	3	3
	AL 42 N=4	IR 42 N=4	CR 42 N=4
Final BW	37.4 $\pm$ 3.1 ^a	30.8 $\pm$ 1.9 ^b	31.1 $\pm$ 3.3 ^b
GU weight	5.4 $\pm$ 0.9 ^a	4.2 $\pm$ 0.9 ^a	3.6 $\pm$ 0.9 ^b
Tumor detection	4	4	3

Across a row mice with different superscript letters are significantly different.

ORIGINAL-TASK 6. Oncogene and tumor suppressor assays.

MONTHS 6-21. Order supplies and set up assays to perform p53, ErbB2 and possibly other growth factors for determination of gene expression and protein levels Complete setting up assays and analyze samples as they become available. (reviewers indicated not to do this)

REVISED-TASK 6. IGF-BP and IGF-I receptors. Since we are focusing on IGF metabolism any work relating to gene expression will concentrate on factors related to IGF-I action such as IGF-I, IGF-I receptors and IGF-BP's.

Progress on Task 6. Molecular biology studies of the tissues obtained will be the focus of study in the following year (we have asked for and been granted a no cost extension). We are still waiting for completion of the pathology from these mice to determine what samples to analyze.

TASK 7. Restock breeding colony.

MONTHS 12-14. Evaluate breeding colony status and initiate breeding for EXPERIMENT 2-FASTING/REFEEDING study.

Progress: This has been completed and we are no longer supporting the breeding colony on this project.

TASK 8. Enroll mice in FASTING/REFEEDING STUDY.

MONTHS 14-21. Breed mice, genotype offspring and enroll mice in FASTING/REFEEDING study. For this study 80-120 mice will be needed depended upon adding a PAIR-FED or a RESTRAINED group. We will have to follow the eating pattern of the FASTING/REFEEDING group for several months to determine if the additional group is needed.

Progress: Due to the longer time frame to enroll the mice in the Intermittent Caloric Restriction studies, mice were not been enrolled in the Fasting/Refeeding study. Also the Hormel Institute instituted Per Diem costs for animal maintenance shortly after this project was funded and it was necessary to pay these costs which has resulted in less money available to carry out these studies. In addition, we did not feel it was prudent to start a new study until we had some idea of what the results of the first study were. Thus due to the much longer time needed for breeding and financial constraints this part of the study was not done.

TASK 9. Serum and tissue analyses and data analyses.

MONTHS 21-24 Complete tissue assays and when all animals are euthanized perform serum analyses and then complete data analyses of EXPERIMENT 1A and 1B.

Progress: IGF-I assays are currently being completed. We have also made arrangements to send the remaining serum samples to University of Pittsburg where Dr. Anna Loshkin will determine insulin, leptin, adiponectin, IL-6 and TNF- α concentrations. We will focus our efforts in the extended year of the project on serum and tissue analyses and continued statistical evaluation of the results.

TASK 10. Manuscript preparation for EXPERIMENT 1A and B.

MONTHS 25-26 Complete manuscript for the first experiment.

Progress: Since data and tissue analyses are underway we have not written any manuscripts. We anticipate starting writing soon and plan to submit abstract(s) for submission to the upcoming AACR Frontiers in Cancer Prevention meeting (November 2006).

TASK 11. Follow mice in FASTING/REFEEDING STUDY.

MONTHS 16-30. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When tumor size dictates kill mice and perform autopsies. Kill nontransgenic mice to be age-matched to mice with tumors. Kill mice upon reaching 50 wk of age if still alive.

Progress: Since we are not pursuing this task there is no progress.

TASK 12. Analysis of tissue samples from FASTING/REFEEDING STUDY.

MONTHS 20-32. Perform assays on tumor and normal tissues from FASTING/REFEEDING STUDY as they become available.

Progress: Since we are not pursuing this task there is no progress.

TASK 13. Serum and tissue analyses of FASTING/REFEEDING STUDY.

MONTHS 28-32. Determine serum analyses from FASTING/REFEEDING as study groups are completed. Complete tissue analysis.

Progress: *Since we are not pursuing this task there is no progress.*

TASK 14. Compete statistical analysis of data from FASTING/REFEEDING STUDY.

MONTHS 33-34. Complete statistical analysis of data obtained from the FASTING/REFEEDING STUDY.

Progress: *Since we are not pursuing this task there is no progress.*

TASK 15. Prepare manuscript from FASTING/REFEEDING STUDY.

MONTHS 35-36. Write manuscript from results obtained from FASTING/REFEEDING STUDY.

Progress: *Since we are not pursuing this task there is no progress.*

KEY RESEARCH ACCOMPLISHMENTS:

Our results now indicate that intermittent caloric restriction provides greater protection against prostate cancer than does chronic restriction. This is very exciting and confirms previous reports from our laboratory for mammary tumor development. We now plan to aggressively pursue identifying tissue characteristics and pathways associated with this protective effect and then to apply for funding to continue these studies.

REPORTABLE OUTCOMES

We plan to submit an abstract for presentation at the AACR 5th Annual Frontiers in Cancer Prevention Conference scheduled for November 2006. We will also begin manuscript preparation shortly.

CONCLUSIONS:

Intermittent caloric restriction regimens may provide a useful tool for prostate cancer prevention. Furthermore, identification of the pathways(s) that mediate this protection should allow for rational drug development for cancer prevention.

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chronic restriction in mouse mammary tumor virus-transforming growth factor- α female mice. *Cancer Epidemiol.Biomarkers Prev.*, **11**, 836-843.

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Personnel receiving pay from the research effort

Margot P. Cleary, Principal Investigator
Michael Grossmann, Research Associate
Patricia Grambsch, Statistician
Olga Rogozin, Research Fellow
Melissa Bonorden, Junior Scientist
Nancy Mizuno, Junior Scientist
Christina Kluczny, summer research student

Animal Care Technicians

Miranda Goff
Laura Hamersma
Michelle Jacobson
Lynn Leraaen
Amy Snider

Appendix 1

REVISED STATEMENT OF WORK PC020457 2/13/03

Eliminating Specific Aim 5 primarily affects Original Task 6. If there is the opportunity to explore tissue analyses as indicated below it will be focused on aspects of IGF metabolism.

TASK 1 & 2. Establish breeding colony & set up genotyping assay.

MONTH 0-3. Order 6 male TRAMP mice (this is the maximum number that can be ordered at one time) and 25 nontransgenic female mice for breeding. Set up breeding. Set up genotyping assay and genotype mice produced. Rebreed mice to expand breeding colony.

TASK 3. Breed mice for EXPERIMENT 1A- LONGITUDINAL STUDY.

MONTHS 4-6. Breed mice to produce one third to one half of mice needed for this study. If one estimates 8 pups per litter, 1 out of 4 pups will be TRAMP males = two TRAMP males per litter. We will need a total of 160 TRAMP males = 80 litters. Genotype offspring. Assign mice to experimental groups. Set up immunohistochemistry assays.

TASK 4. Complete enrollment of mice for EXPERIMENT 1A and 1B SERIAL STUDY

MONTHS 7-12. Continue breeding to complete EXPERIMENT 1A. Three to four rounds of breeding will probably be needed to supply enough mice. Genotype mice as they are produced. Assign mice to experimental groups. Once longitudinal study is complete begin assigning mice to serial study for EXPERIMENT 1B.

TASK 5. Follow mice in EXPERIMENT 1A and 1B.

MONTHS 6-21. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When age, tumor size and/or animal condition dictates euthanize mice and perform autopsies. Euthanize nontransgenic age-matched mice to correspond to those TRAMP mice with tumors. Euthanize mice that reach terminal ages of 48 or 50 wk of age. Record results and when study complete do statistical analyses of results.

ORIGINAL-TASK 6. Oncogene and tumor suppressor assays.

MONTHS 6-21. Order supplies and set up assays to perform p53, ErbB2 and possibly other growth factors for determination of gene expression and protein levels. Complete setting up assays and analyze samples as they become available.

REVISED-TASK 6. IGF-BP and IGF-I receptors. Since we are focusing on IGF metabolism any work relating to gene expression will concentrate on factors related to IGF-I action such as IGF-I, IGF-I receptors and IGF-BP's.

TASK 7. Restock breeding colony.

MONTHS 12-14. Evaluate breeding colony status and initiate breeding for EXPERIMENT 2-FASTING/REFEEDING study.

TASK 8. Enroll mice in FASTING/REFEEDING STUDY.

MONTHS 14-21. Breed mice, genotype offspring and enroll mice in FASTING/REFEEDING study. For this study 80-120 mice will be needed depended upon adding a PAIR-FED or a RESTRAINED group. We will have to follow the eating pattern of the FASTING/REFEEDING group for several months to determine if the additional group is needed.

TASK 9. Serum and tissue analyses and data analyses.

MONTHS 21-24 Complete tissue assays and when all animals are euthanized perform serum analyses and then complete data analyses of EXPERIMENT 1A and 1B.

TASK 10. Manuscript preparation for EXPERIMENT 1A and B.

MONTHS 25-26 Complete manuscript for the first experiment

TASK 11. Follow mice in FASTING/REFEEDING STUDY.

MONTHS 16-30. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When tumor size dictates kill mice and perform autopsies. Kill nontransgenic mice to be age-matched to mice with tumors. Kill mice upon reaching 50 wk of age if still alive.

TASK 12. Analysis of tissue samples from FASTING/REFEEDING STUDY.

MONTHS 20-32. Perform assays on tumor and normal tissues from FASTING/REFEEDING STUDY as they become available.

TASK 13. Serum and tissue analyses of FASTING/REFEEDING STUDY.

MONTHS 28-32. Determine serum analyses from FASTING/REFEEDING as study groups are completed. Complete tissue analysis.

TASK 14. Complete statistical analysis of data from FASTING/REFEEDING STUDY.

MONTHS 33-34. Complete statistical analysis of data obtained from the FASTING/REFEEDING STUDY.

TASK 15. Prepare manuscript from FASTING/REFEEDING STUDY.

MONTHS 35-36. Write manuscript from results obtained from FASTING/REFEEDING STUDY.