

UNIVERSITY OF KENTUCKY
Internal Approval Form

Revised 3/96

ONLY NOTED (●) ITEMS MUST BE COMPLETED FOR NONCOMPETING OR CONTINUATION PROPOSALS UNLESS THERE ARE CHANGES FROM THE ORIGINAL APPROVED PROPOSAL.

● Principal Investigator: Geza Bruckner E-mail Address: gbruckn@pop.uky.edu Social Security No.: _____
 Dept: Clinical Sciences College/Center or Institute: Allied Health Campus Phone: 31100-246
 Physical location of Proposed Activity (Building): MDR3 FAX: 2571816
 If the unit primarily responsible for this proposal is other than the prime department of the PI, specify alternate unit: _____
 Co-Principal Investigator: _____ E-mail Address: _____ Social Security No.: _____
 Dept: _____ College/Center or Institute: _____ Campus Phone: _____
 Co-Principal Investigator: _____ E-mail Address: _____ Social Security No.: _____
 Dept: _____ College/Center or Institute: _____ Campus Phone: _____
 Attach additional sheets if necessary

● Title of project: Lipid metabolism in feline Hepatic Lipidosis: impact of taurine and carnitine
 Sponsoring Agency: IAMS Co. Phone: 937-415-8908
 Address: Lewisburg, Ohio Contact: Dr. Greg Sunvold FAX: 937-415-8708
 ● Agency Deadline Date: ____/____/____ Postmark Receipt No Specific Deadline

● Current Budget Period from: 06 / 1 / 99 to 05 / 30 / 01 Total Project Period from: 06 / 1 / 99 to 05 / 30 / 01
 ● Current Period Request: \$ _____ Total Project Request: \$ 141,866

Check one box in each section:

● Proposal is:	Activity is:	Activity is:
<input checked="" type="checkbox"/> New	<input checked="" type="checkbox"/> Applied Research	<input type="checkbox"/> Conference
<input type="checkbox"/> Competing Renewal	<input checked="" type="checkbox"/> Basic Research	<input type="checkbox"/> Equipment Request
<input type="checkbox"/> Noncompeting Renewal or continuation of Project Act: _____	<input type="checkbox"/> Clinical Research	<input checked="" type="checkbox"/> Instruction
<input type="checkbox"/> Supplemental	<input type="checkbox"/> Post Doc Research Training	<input type="checkbox"/> Research
<input type="checkbox"/> Response to RFP/RFQ # _____	<input type="checkbox"/> Graduate Training	<input type="checkbox"/> Public Service
	<input type="checkbox"/> Undergrad Training	<input type="checkbox"/> Other sponsored activity
	<input type="checkbox"/> Continuing Educ	
		<input type="checkbox"/> Specify _____

If this project qualifies as a "Special Purpose or Circumstances" per University Costing Guidelines, indicate the Special Purpose #: _____

● Does This Project Involve Any of the Following?

1. Human Subjects	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Date Approved: ____/____/____	IRB#: _____
2. Animal Subjects	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Date Approved: <u>10 / 16 / 98</u>	IACUC#: <u>960063M</u>
3. Hazardous Materials	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
4. Radioactive Materials	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		

Will this project require any of the following?

1. Purchase of Additional Equipment If yes, has University inventory been screened for availability of existing equipment?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
2. Additional University Space? Will any part of the project be conducted in non-University facilities?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	If yes, attach explanation
3. Alterations or Renovations of Existing Facilities?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	If yes, attach explanation
4. Faculty or Staff Overload?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	If yes, attach explanation
5. Subcontracted or Outside Consultants?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	If yes, attach explanation



List up to 5 keywords to describe this project Other Than words contained in the title

1. _____ 2. _____ 3. _____ 4. _____ 5. _____

Check All areas which describe this project

- | | | | |
|--|---|--|--|
| <input type="checkbox"/> Aging/Gerontology | <input type="checkbox"/> Cardiovascular/Respiratory | <input type="checkbox"/> Health Care Financing | <input type="checkbox"/> Population |
| <input type="checkbox"/> Agriculture | <input type="checkbox"/> Cellular/Molecular Biology | <input type="checkbox"/> International | <input type="checkbox"/> Rehabilitation/Disengineering |
| <input type="checkbox"/> Alcohol/Drug Abuse | <input type="checkbox"/> Coal | <input type="checkbox"/> Mental Health | <input type="checkbox"/> Public Service |
| <input type="checkbox"/> Appalachia | <input type="checkbox"/> Crime/Justice | <input type="checkbox"/> Mining/Minerals | <input type="checkbox"/> Tobacco |
| <input type="checkbox"/> Biotechnology | <input type="checkbox"/> Energy | <input type="checkbox"/> Minorities | <input type="checkbox"/> Toxicology |
| <input type="checkbox"/> Business/Industry Cooperation | <input type="checkbox"/> Environment | <input type="checkbox"/> Neuroscience | <input type="checkbox"/> Transportation |
| <input type="checkbox"/> Cancer | <input type="checkbox"/> Family | <input type="checkbox"/> Nutrition | <input type="checkbox"/> Women |

PROPOSAL BUDGET SUMMARY

ALL costs of a project are to be reimbursed by the sponsoring agency, all proposals must include a complete budget reflecting full costs of the project; any cost not reimbursed by the sponsor must be clearly indicated along with the internal source of funds to cover them.

If it is known that this project will be funded by a fixed-price agreement, complete Column A of a Fixed-Price Agreement Budget Form in lieu of this budget summary, and attach the form.

● BUDGET PROPOSED IN ATTACHED PROPOSAL

	Sponsor	University	Total
Personnel	53,120	0	53,120
Other Direct Costs	0	0	0
Equipment	70,242	0	70,242
Indirect Costs	18,504	0	18,504
TOTAL	141,866	0	141,866

If any amounts appear in the "University" column explain why you are not requesting the sponsor cover full cost of the project. If the sponsor will not pay full indirect costs attach a copy of their written policy. The sponsor in the past has only paid 10% but it is anticipated that 15% might be covered

Indicate the specific source of any University funds to be used to conduct this project _____

For the purpose of allocating actual indirect cost/incentive funds to be generated by this project, indicate the proportion of total incentive funds to be applied to each department or center/institute. Percentages should reflect the relative contributions (investigator time, facilities, cost-sharing, etc.) of each unit involved.

Medical Center Prehospital Unit	Unit	Clinical Sciences	100%
	Unit		%
	Unit		%
	TOTAL		100%

CERTIFICATION AND SIGNATURES

There must be signature approvals from all departmental chairs, deans or directors, and chancellors or vice presidents whose personnel or facilities are involved in conducting the proposed work.

INVESTIGATORS' CERTIFICATION: My signature below indicates that 1) I am not presently debarred or suspended from receiving federal funds, 2) no federal funds were used for lobbying activities in connection with this proposal, 3) I am not delinquent on any federal debt, 4) the budget above represents the best estimate of the full costs of the project and identifies all sources of funds to cover full costs, and 5) in the conduct of the proposed project I will adhere to University policies including conflict of interest, ethical standards in the conduct of research, intellectual properties and the use of humans and animals in research. **I have completed a "Disclosure of Financial Interest" form and submitted it to my dean/director/CCS President**

CHAIRS' AND DEANS/DIRECTORS/CCS PRESIDENTS' CERTIFICATION: We certify that we have reviewed the proposal, including the full cost budget and sources of internal funds, and that it is consistent with the educational and research objectives of the unit. We also agree with the above distribution of indirect cost. **Deans/Directors/CCS Presidents have received completed "Disclosure of Financial Interest" forms from each investigator in their college/center. Please attach signed form whenever possible.**

INVESTIGATOR(S)	CHAIR	DEAN/DIRECTORS/CCS PRESIDENT	CHANCELLOR/VP
PI <u>[Signature]</u> Date <u>5/25/99</u>	<u>[Signature]</u> Date <u>5/25/99</u>	<u>[Signature]</u> Date <u>5/25/99</u>	<u>[Signature]</u> Date <u>11/2/99</u>
CO-PI Date	Date	Date	Date
CO-PI Date	Date	Date	Date

UNIVERSITY OF KENTUCKY
Staff/Faculty Disclosure of Financial Interest Form

This form must be completed by all grant applicants (including the principal investigator, any co-principal investigators, and any other University employee who is or will be responsible for the design, conduct or reporting of activities under the sponsored project). This form must be submitted to your dean or director before the University can submit an application for any sponsored research agreement, and prior to registration or transfer of technology arising out of any faculty or staff member's research.

Name: Geza Bruckner Date: 5/25/99

Title & Position: Professor College: Allied Health

Social Security Number: _____ Dean or Director: Tom Robinson

31100-246 Geza Bruckner

Campus Telephone Number _____ Principal Investigator

Proposal Title: Lipid metabolism in feline hepatic lipidosis: Impact of taurine etc.

Sponsor: IAMS Proposal Deadline: none

This Financial Disclosure Statement is to be completed in connection with the University's Conflict of Interest/Financial Disclosure Policy—Research, AR II-4.0-4. Please make reference to the regulations for definitions of the terms used herein.

Please note, answering "yes" to any of the following questions does not mean the financial interest is inappropriate or improper, it means only that disclosure and evaluation, and in some cases, approval and oversight, are required. For further information, refer to AR II-4.0-4

The purpose of this Financial Disclosure Statement in conjunction with the University's Policy on Financial Conflict of Interest in Research is to help investigators determine if the combinations of their professional activities pose any threat to the objective pursuit of their research.

1. Financial Compensation from Related Business YES NO

Are you, or is anyone in your immediate family, currently receiving income from a business in any way related to or that might be affected by your proposed sponsored research activities or transfer of technology (such as consulting or other fees; salary; allowance, dividend; rent; capital gain; real or personal property), and that when aggregated over the next twelve months is expected to exceed \$10,000?

2. Equity Interest in Related Businesses YES NO

Are you, or is anyone in your immediate family, currently holding financial interests exceeding \$10,000 or 5% ownership in a business enterprise related to your proposed research activities or transfer of technology?

Continued



3. Intellectual Property and Related Businesses YES NO

Are you, or is anyone in your immediate family, currently entitled to receive compensation from a business enterprise due to intellectual property in any way related to or that might be affected by your proposed research or transfer of technology (such as patents, copyrights, or royalties agreements)?

4. Other Relevant Financial Interests YES NO

Are there any other situations not covered above might possibly be affected by the research for which funding is sought?

If you answered YES to any of the above questions, please attach a complete description of the situation. (You need not disclose dollar amounts.)

I have read and understood the University of Kentucky's Policy on Conflict of Interest/Financial Disclosure-Research; have made all required financial disclosures; will submit a proposal for a conflict management plan if necessary; and will comply with any conditions or restrictions imposed by the University to manage, reduce, or eliminate conflicts of interest regarding my research.

[Signature]
Signature

5/25/99
Date

TO BE COMPLETED BY DEAN OR CENTER OR INSTITUTE DIRECTOR/
COMMUNITY COLLEGE PRESIDENT:

Disclosure form reviewed by Wm Pfeiffer as Assoc. Dean on 25 May 1999

Were any potential conflicts noted?

YES

NO - See attached STATEMENT

If NO, forward this form in a sealed envelope marked "confidential" to the Office of Sponsored Projects Administration, Second Floor, Kinkead Hall 0057.

If YES, refer to AR II-4.0-4. You and the researcher are to propose a joint plan of management to submit to the Vice President for Research and Graduate Studies.

[Signature]
Signature: Dean or Director

IF POSSIBLE, PLACE THIS COMPLETED FINANCIAL DISCLOSURE FORM IN A SEALED ENVELOPE AND ATTACH TO THE INTERNAL APPROVAL FORM.

RESEARCH PROPOSAL

Title Page

Title of Research Project: Lipid Metabolism in Feline Hepatic Lipidosis (FHL): Impact of Taurine and Carnitine .

Project Objectives: Feline hepatic lipidosis is a well-recognized hepatopathy which is characterized by extensive lipid accumulation. Although diabetes mellitus and acute pancreatitis appear to contribute to the pathogenesis of FHL, the majority of cases are believed to result from the nutritional and biochemical peculiarities of the cat^{1,2} which to date are not understood. **Therefore, the broad long term objectives of our continued studies in obese cats undergoing rapid weight loss are to:**

1. **Determine how dietary taurine impacts on the etiology of FHL.**
2. **Determine how dietary carnitine impacts on the etiology of FHL.**
3. **Determine how dietary taurine interacts with carnitine and it's impact on FHL.**

Impact of the Proposed Studies on the Nutritional Health of Cats: FHL is the most common hepatopathy encountered in cats and its pathogenesis remains unknown. The mortality rate in cats even with aggressive nutrition therapy approximates 40% and approaches 90% in untreated cats. Our ongoing studies will help to elucidate the basic mechanisms involved in feline lipid metabolism (**fatty acid oxidation, fatty acid turnover** and lipoprotein transport) and contribute to elucidating hepatic dysfunction associated with FHL. The proposed studies, **based on our current results**, are designed to test the impact of **taurine and carnitine or their interactions** on the mechanism(s) associated with the development of FHL. Overall, the proposed studies may lead to the development of specific feline diets which will prevent the onset of FHL and/or facilitate the recovery of cats with FHL (particularly during stress related weight reduction in obese cats).

Principal Investigator: Geza G. Bruckner, Ph.D. Professor/Division Director Clinical Nutrition, Department of Clinical Sciences, University of Kentucky, Lexington, Ky.

Grant Amount Requested: Total Budget Request for 2 Year Period = \$141,866

RESEARCH PROJECT PROPOSAL

Expanded Research Objectives:

Cats would be spayed and fed a high caloric diet **low in LPUFAs and deficient in taurine and marginal in choline and methionine** until they gained at least 30% above their lean bodyweights, thus minimizing stores of these nutrients. A modification of the obese/low calorie weight reduction feline model, as we have recently used and which was developed by Biourge et al.³, will be used to assess the impact of the various nutrient interactions as they impact the development of FHL. During the rapid weight reduction period diets would be provided which are sufficient or deficiency in taurine and/or carnitine. These diets would be provided at 25% of lean bodyweight maintenance energy (ME) with vitamins and minerals supplemented at 4X NRC. Triglyceride turnover rates would be assessed using C13 glycerol. Ketone body formation and lipoprotein synthesis would be determined using C13 palmitate. Body weight changes would be recorded weekly. Liver biopsies would be performed to evaluate liver lipid scores and peroxisomal and mitochondrial structure using light and electron microscopic techniques.

Specific Aims:

1. **Elucidate which of the mechanism(s) in lipid and lipoprotein metabolism are altered by carnitine and/or taurine in feline hepatic lipidosis (FHL).** Specifically, we will determine the rate of stable isotope incorporation (labeled palmitate) into very low density lipoprotein (VLDL) in plasma, liver triglycerides as well as **ketone body** formation of cats fed different diets formulated to be sufficient or deficient in **taurine and/or carnitine**. Our previous experiments using deuterium incorporation suggested that no new fatty acid synthesis or incorporation of these FA into VLDL occurred during the weight reduction period. However, these studies did not assess the potential of preformed fatty acid to be incorporation into VLDL or triglycerides.
2. **Determine the ultra structural changes in liver mitochondria and peroxisomes in cats fed the different diets as related to development of FHL.** Specifically we will focus on ultrastructural changes using light and electron microscopy in both peroxisomes and mitochondria and correlate these changes with the ability of these organelles to β oxidize fatty acids.

EXPECTED OUTCOME:

Using a modification of the obese/low calorie weight reduction feline model, we anticipate, based on our previously reported findings, identifying the mechanism(s) which are responsible for induction of fatty livers and the role that taurine and carnitine specifically contribute to peroxisomal and mitochondrial alterations in FHL.

We have found that the absence of LPUFAs and the protein quality of the cat's diet contributes to the development of FHL, and it is likely that **phospholipids** formed from LPUFAs are rapidly depleted or the synthesis rate is reduced in cats undergoing a modified fast. Additionally, since a strong correlation has been found between taurine and phospholipid methylation rates in synaptosomes, it is possible that taurine, which is essential to the cat, plays a pivotal role in phospholipid biosynthesis (See Fig below). We speculate that low levels of dietary taurine combined with low choline intake contribute directly to peroxisomal and mitochondrial membrane function thereby altering fatty acid transport and/or fatty acid β oxidation. We speculate that this alteration in peroxisomal and mitochondrial function are the primary cause of FHL.

We anticipate that the absence of **taurine and carnitine**, against a background of marginal choline, methionine and LPUFA's, will rapidly precipitate the development of FHL. The inclusion of taurine and/or carnitine in the diet will prevent the development of FHL with a protective effect in the following order: **taurine&carnitine > taurine > carnitine**.

BACKGROUND INFORMATION

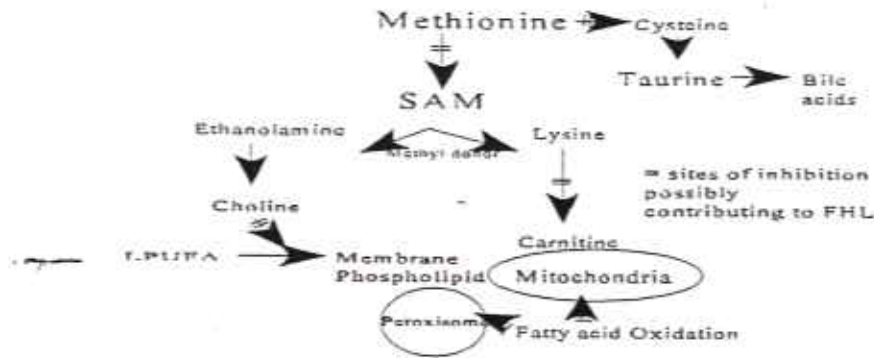
Feline hepatic lipidosis is a well-recognized hepatopathy which is characterized by extensive lipid accumulation. Although diabetes mellitus and acute pancreatitis appear to contribute to the pathogenesis of FHL, the majority of cases are believed to result from the nutritional and biochemical peculiarities of the cat^{4,5}. Many but not all cats are obese at the onset of the disease with anorexia being a common denominator. Stressor events are associated with the onset of the disease and a common stressor appears to be dietary change to less palatable diets such as noted for weight reduction diets⁶. Serum biochemical parameters usually show evidence of cholestatic disease indicated by elevations in total serum bilirubin and alkaline phosphatase. Although high protein cat diets, human enteral products and veterinary enteral products have been used in the treatment of FHL, it is apparent that there is less than an ideal prognosis with an approximate 40-50% current mortality rate². The ideal diet is yet to be formulated. Better diet formulation is unlikely until the etiology of the disease is understood. Cats, unlike most other pets, develop hepatic lipidosis during a fast. Based on our recent studies (to be published) the most likely mechanism(s) involved in the development of FHL are decreased mitochondrial and/or peroxisomal fatty acid oxidation.

The dietary requirements of the cat appear to make it more susceptible to hepatic lipidosis than other species. The cat has very minimal $\Delta 6$ desaturase enzyme activity⁷ and therefore common vegetable oil sources, such as corn oil, appear to be inadequate for meeting its essential fatty acid requirement⁸. Essential fatty acid deficiency is known to induce fatty livers in cats⁹ and other animal models^{10,11}. Essential fatty acid deficiency (EFAD) is also known to effect lipoprotein lipase, lecithin cholesterol acyltransferase, and fatty acid synthetase activities¹². There is also an apparent defect in lipoprotein transport from the liver of EFAD animals¹³. Based on these previously reported observations, it is plausible that the feline is more susceptible to EFAD and the associated changes in lipid metabolism. Diets which have recently been used to induce and study FHL are EFAD, most likely even when supplemented with corn oil^{1,13} (based on the above cited literature and, **data from our recent study, which was presented at the Iams symposium and has recently been submitted for publication (still pending), shows that levels of 20:4n6 and 22:6n3 are reduced in the liver of cats undergoing rapid weigh loss when fed a corn oil based diet. Furthermore these fatty acid changes are impacted by protein quality suggesting that desaturase activity may be further compromised.**

Dietary protein is important in the management of FHL and it has been shown that feeding 25% of the maintenance energy (ME) as high quality protein will attenuate the hepatic lipidosis but does not ameliorate the condition (reduced liver lipid content by approx 50% compared to corn oil supplemented animals)¹³. A poor quality protein source (corn gluten meal) appears to contribute to the development of FHL by perhaps decreasing the already low activity of key desaturase enzymes and thus lowering the levels of membrane LPUFAs (**results of our recent study**). It has been shown in numerous animal species that a methionine deficiency contributes to the development of hepatic lipidosis but little is known about the impact of dietary taurine on phospholipid metabolism.

It is speculated that both methionine and taurine are involved in the methylation of phospholipids¹⁴. Taurine may also contribute to membrane alterations as a scavenger of free radicals¹⁵ or as a membrane stabilizer¹⁶. Also taurine has been shown to reduce liver lipid content in obese children¹⁷. With a poor quality protein source, which is low in methionine, the synthesis of carnitine may also be decreased and subsequently fatty acid transport into the mitochondria and oxidation may be impaired.

Possible pathways involved in the pathogenesis of FHL



EXPERIMENTAL DESIGN

Objectives 1,2,3 Using a modification of the obese/low calorie weight reduction feline model (retired breeder, purpose breed, spayed cats) and a 2X2 factorial design, we will test the effects of taurine and carnitine supplementation in diets which will be fed at 25% of ME, with 4X the NRC requirements for vitamins and minerals, on the development of FHL.

(- taurine, - carnitine) Corn gluten meal (or other protein source low in methionine) plus oil blend n=6	(- taurine, + carnitine) Corn gluten meal (supplemented with carnitine) plus oil blend n=6
(+ taurine, - carnitine) Corn gluten meal (supplemented with taurine) plus oil blend n=6	(+ taurine,+ carnitine) Corn gluten meal (supplemented with taurine and carnitine) plus oil blend. n=6

Animals For Dietary Studies Twenty-four female cats, retired breeders (purpose breed), will be used for the study. Cats will be anaesthetized (ketamine and isoflurane) and spayed; blood samples (10ml) and a wedge liver biopsy will be taken during surgery (all surgical procedures and protocol will be carried out according to the "Guide for the care and use of laboratory animals" and IACUC approved). Cats will be fed an energy dense diet developed by IAMS (see below under Diets). Diets will be fed *ad libitum* until they gain a minimum of 30% over their lean body weights compared to healthy lean cats of the same length and body type (estimated 2-3 months). Once the animals have attained 30+% obesity they will be assigned randomly to the four treatment groups (6 animals/trt) in staggered intervals (4 animals/week; 1/each trt/week). The cats would be maintained on the weight reduction diets for 7-8 weeks or until they reach body weights similar to but not less than -10% of the bodyweight established for healthy cats of the same body type and length. Other limitations to the fast would include bilirubin levels >0.4 mg/dl.

Diets All of the diets to be used would be formulated and provided by IAMS. Spayed cats will be fed an energy dense, highly palatable diet developed by IAMS to be low (or devoid) in LPUFAs. The diets will also be formulated to contain marginally sufficient amounts of choline and methionine. Once the cats have reached the 30+% overweight status, weight reduction dietary regimens would be initiated. The composition of the four weight reduction diets would be formulated by IAMS and would be formulated in a similar fashion to the previously used

diets*.

*micro-nutrients supplemented at 4X the required daily amounts so that diets provided at 25% of ME (ME kcal = $1.4 \times (70 + 30 [\text{body weight in kg}])$) will provide 100% of all vitamins and minerals.

Spayed obese female retired breeders will be fed the diets described above at 25 % of ME on a daily basis. Water will be provided to all cats *ad libitum* and a 12 hr dark/light cycle maintained.

Experimental Protocol (time table)

The anticipated time frame for the protocol is as follows: 1) Procurement of twenty-four retired breeder female cats will take 3-4 months (Harlan can supply 7-9 cats/month, Liberty about 4-6 cats/month), 2) The weight gain period will be between 3-4 months depending on the initial adiposity of the animal, 3) Completion of the weight reduction period for all staggered treatment groups will be about 14 weeks based on our previous studies; four animals/week will be randomly assigned to the 4 treatment groups ($24 \text{ animals} \div 4 \text{ animals/week} = 6 \text{ weeks}$) and each group will be on the weight reduction regimen for approximately 7 weeks.

/ Procurement of cats			/Weight gain period				/ Dietary treatments				/Assays Completed			/ Stat.&Data Anal & Publish			
1	2	3	4*	5	6	7	8	9	10	11	12	13	14	15	16	17	18-24
			a,b,c	c	c	a,b,c	a	c	a,b	a,b,c							
months																	

* = animals spayed.

a = blood sample for lipoproteins, glucose, free fatty acids, insulin, glucagon, bilirubin, vet panel, ketone bodies, etc.

b = liver biopsy for histology, electron microscopy, fatty acid and triglyceride assessment.

c = weighed weekly.

d = liver resect on 4 animals/week for isotopomer studies.

e = DEXA

Measurements and Collections

Blood samples (10 ml/collection period) will be collected from the jugular vein of anaesthetized cats. Samples will be drawn into EDTA, heparin or glass tubes depending on the sample needed (plasma or serum).

Liver biopsies will be performed as a wedge biopsy for the initial sample during the spaying procedure, thus minimizing the number of invasive procedures required. Subsequent wedge biopsies at the end of weight gain and weight loss periods will be done using a combination of acetylpromazine, xylazine and ketamine anaesthesia. A portion of the samples will be fixed in 10% buffered formalin, stained with osmium tetroxide for neutral lipid, imbedded in paraffin sectioned and counter stained with H&E¹⁸. Slides will be digitized using a Zeiss microscope connected to image processing software (NIH IMAGE 1.60). Threshold optical density values set to blank out non-specific staining. Optical density units thus directly correlate with the intensity of neutral lipid staining and are expressed as % optical density/mm². Values will also expressed on a 1 to 6 scale with 1 to 2 considered normal, 3 to 4 having mildly increased lipid staining, 5 showing definite lipid accumulation and 6 equal to severe lipodosis; all samples will be assessed with the operator blinded to the sample origin. Electron microscopic analysis of liver samples will also be conducted. Tissue samples (1 mm³) will be immersed in a solution containing 4% paraformaldehyde and 0.5% glutaraldehyde in 0.14 mol/l phosphate buffer, at pH 7.0, for several weeks. After thorough washing in the same buffer, the samples will be postfixed in 2% osmium tetroxide, dehydrated in a series of ethanol, then embedded in Durcupan resin. Sixty nm ultra-thin sections will be cut with a diamond knife using a Reichert-Jung Ultracut E ultramicrotome. The ultra-thin sections will be contrasted with lead citrate and uranyl acetate, then viewed, and photographs taken using a JEOL 200CX electron microscope. The sections will be analyzed as follows: 10 cell profiles/section/sample will be counted and the number of lipid inclusions, mitochondria and peroxisomes (small, round, about 250-500 nm in diameter, with crystalloid inclusions) will be determined/cell profile. The average for all profiles will be calculated. Additionally, peroxisome and mitochondrial structures will be fully characterized.

Vet Panel 1 will be done on serum samples to determine albumin, alkaline phosphatase, total bilirubin, cholesterol, glucose, protein, SGOT, BUN and calcium (*samples sent to LAMS* - results immediately sent back to investigator to monitor liver function).

Insulin and glucagon will be determined by *LAMS* using standard RIA.

Lipoprotein (VLDL) will be measured using a modification of our current procedure which is based on that reported by Chung et al.¹⁹ and the procedures reported by Dimski et al.²⁰.

Fatty acid analysis will be performed on liver tissue samples with emphasis on changes in triglyceride and phospholipid essential fatty acids.

Isotopomer spectral analysis will be utilized to determine the rate of a) β -hydroxybutrate (fatty acid oxidation index) and b) VLDL synthesis in plasma and TG synthesis in the resected liver samples using C13 palmitate as described by Kelleher et al.^{21,22,23}. C13 glycerol will be used to determine triglyceride turnover in adipose tissue.

Free fatty acids will be determined in the plasma using methods described by Ingalls et al.²⁴.

DEXA Dual energy x-ray absorptiometry would be used to determine body composition following weight gain and weight loss.

Statistical Analysis Data will be analyzed by two way analysis of variance (ANOVA) followed by least square means analysis (LSM) to measure significant differences between treatment groups.

Itemized Budget For 2 Year Period

Personnel		
Post Doc Wissam Ibrahim 100% (Salary and benefits) (1.5 years)	42,120	
Responsible for conducting all aspects of the study in collaboration with Dr. Bruckner (Bruckner 5% time allocated to project)		
Graduate student (50%)		
Responsible for isotopomer spectral analysis as it relates to GC/MS and data analysis		
	8,000	
Consultant, data analysis and manuscript prep (Dr. Szabo) 1 mo/yr	3,000	
Animals and Care		
Cats (Total 24 @ \$500/cat)	12,000	
Cats Per Diem		
24 weight gaining cats x 100 days x \$5.50/day=	13,200	
24 obese weight/reduction cats x 56 days x \$5.50/day=	7,392	
24 cats spaying and 2 surgeries @ \$200/event	14,400	
Lab Supplies		
DEXA (\$100 x 24 x 2)	4,800	
Liver electron micrographs (\$150 x 24 x 2)	7,200	
Lipid reagents, TLC, Sep-paks, tubes, etc.	3,000	
C13 isotope and supplies for GC/MS	5,500	
Vet Panel 1 (4 samples/cat x 24 cats, to be <i>done by LAMS</i>)	-	
Insulin/glucagon (4 samples/cat x 28 cats, to be <i>done by LAMS</i>)	-	
Miscellaneous, long distance calls, paper, supplies, etc.	1,500	
Shipping of plasma samples to IAMS	250	
Travel to present data at EB meeting	1,000	
Sub-Total	\$ 123,362	
Indirect @ 15%	18,504	
Total	\$141,866	

1. Zawie DA, Garvey MS. Feline hepatic disease. *Vet Clin North Am Small Anim Prac* 14:1201-1230, 1984.
2. Dimski DS, Taboada J. Feline Idiopathic hepatic lipidosis. *Vet Clin North Am Small Anim Prac*. 25:357-373, 1995.
3. Biourge VC, Groff JM, Munn RJ, et al.: Experimental induction of hepatic lipidosis in cats. *Am J Vet Res* 55:1291-1302, 1994.
4. Zawie DA, Garvey MS. Feline hepatic disease. *Vet Clin North Am Small Anim Prac* 14:1201-1230, 1984.
5. Dimski DS, Taboada J. Feline Idiopathic hepatic lipidosis. *Vet Clin North Am Small Anim Prac*. 25:357-373, 1995.
6. Cornelius L, Rogers K. Idiopathic hepatic lipidosis in the cat. *Mod Vet Pract*. 66:377-380, 1985.
7. Pawlosky R, Barnes A and Salem N. Essential fatty acid metabolism in the feline: relationship between liver and brain production of long-chain polyunsaturated fatty acids. *J Lipid Res*. 35:2032-2040, 1994.
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CONFIDENTIAL



November 5, 1999

Dr. Geza Bruckner
University of Kentucky
Division Clinical Nutrition
College of Allied Health
121 Washington Avenue
Lexington, KY 40506-0080

Dear Dr. Bruckner:

The Iams Company is pleased to have the opportunity to support the collaborative research study involving the role of carnitine and taurine in feline hepatic fatty acid metabolism.

The total contracted amount of support for this study is \$141,600. **The initial payment of \$56,600 was issued and forwarded to your attention in October, 1999.** An intermediate payment allocation of \$56,600 will be made available in September, 2000, and the final balance of \$28,400 will be issued approximately September, 2001. The date of the final payment is dependent upon the status of the research study at that time. Additionally, The Iams Company requires the submission of a project summary or manuscript draft before the final balance is made available to the University of Kentucky.

The Iams Company appreciates your efforts during this research study. Please let me know if you have any questions regarding the support of this program.

Sincerely,

A handwritten signature in black ink, appearing to read "Gregory D. Sunvold".

Gregory D. Sunvold, Ph.D.
Manager, Clinical Research
Research & Development

dms:gds991104-buster.doc1



6571 St. Rt. 503 North
P. O. Box 189
Lewisburg, OH 45338-0189
(937) 415-8801
Fax (937) 415-8708

Ear tag #	Tattoo#	Bruckner's #	Name	Owner	Declaw	Liver Bx	Interviewed	Home
✓4776	H5K299	30	Sarah		front	08/15/00	8/16/2000	8/25/00
-4777	H5J275	31	Miranda		front	08/02/00	7/31/2000	8/22/2000
✓4778	H5L279	32	Gracie		no	08/15/00	9/5/00	9/5/00
✓4779	H6A043	33	Millie		no		8/23/2000	8/23/2000
✓4780	H5G065	34	Cally		no	08/10/00	8/24/2000	
✓4781	H5D067	35	Rose		front	08/23/00	8/23/00	
✓4782	H5J215	36	Agatha		no	08/23/00	9/5/00	9/5/00
✓4783	H5G063	37	Francesca		no	08/23/00	8/31/00	9/1/00
✓4784	H5G151	38	Marilyn		no	08/02/00	8/9/2000	8/11/2000
✓4785	H5C121	39	Elizabeth	Died	no	08/23/00	XXX	XXX
✓4786	H4A137	40	Pandora		no	08/23/00	8/30/00	
✓4787	H5I271	41	Madeline	not adopting out		08/02/00	XXX	XXX
✓4788	H5I377	42	Sally		front	08/15/00	8/16/2000	8/25/00
✓4789	H5L015	43	Katie		front	08/15/00	8/29/00	8/31/00
✓4790	H4O155	44	Susan	not adopting out			XXX	XXX
✓4791	H4B017	45	Rachel		front	08/23/00	8/11/2000	
✓4792	H6A045	46	Carmen		no	08/10/00	8/22/2000	8/22/2000
✓4793	H5B143	47	Charlotte		no	08/02/00	7/31/2000	8/11/2000
✓4794	H5I143	48	Tessa		no	08/02/00	8/9/2000	8/11/2000
✓4795	H5J143	49	Evie		front	08/02/00	7/26/2000	8/18/2000
✓4796	H5M225	50	Isabel		front	08/15/00		8/25/00
✓4797	H5F033	51	Cleopatra		no	08/10/00		
✓4798	H4B021	52	Sophie		no	08/10/00	8/16/2000	8/18/2000
✓4799	H4D249	53	Debbie	not adopting out		08/15/00	XXX	XXX

All cats adopted except for the following:

- ✓4785 — Liver biopsy 6/22/00, bit PW on 1/27/00 — Found dead 8/23/00
- ✓4790 — Euthanatized at biopsy 8/10/00 because of grossly large spleen
- ✓4799 — Died during prep for biopsy 8/15/00, necropsy revealed diseased kidneys
- ✓4787 — Biopsy 6/20/00, euthanatized because of indications renal disease (high creatinine)



Protocol: 990069M

000002545

PI: Bruckner, Geza

Notify: Bruckner, Geza (606) 233-5898

44

Strain: Cat (Domestic)

Age: Retired Breeder

DOB: 12/15/94

Sex: F

Weight: 2.47 Kg

ID: 4790

H40155

Vendor:

Q1/24/2000

Req. #: A00553:2

count: 463462

Euth

Euthenatized



Protocol: 990069M

000002549

PI: Bruckner, Geza

Notify: Bruckner, Geza (606) 233-5898

53

Strain: Cat (Domestic)

Age: Retired Breeder

DOB: 4/23/94

Sex: F

Weight: 2.97 Kg

ID: 4299

H4-D249

Vendor:

Q1/24/2000

Req. #: A00553:2

count: 463462

Died - anesthesia JSK 8/15/00

Died under anesthesia



0000002541

Protocol: 990069M

PI: Bruckner, Geza

Notify: Bruckner, Geza (608) 233-5698

Strain: Cat (Domestic)

Age: Retired Breeder

Weight: 2.43 kg

Vendor:

Received: 01/24/2000

DOB: 01/28/95

Sex: F

ID: 1727

41
431271

Req. #: A00553:2

count: 463462

Madeline

4787

8/2/00

*Euthanized by PI -
High Creatinine*



0000001659

Protocol: 990069M

PI: Bruckner, Geza

Notify: Bruckner, Geza (608) 233-5698

Strain: Cat (Domestic)

Age: Retired Breeder

Weight:

Vendor:

Received: 01/19/2000

DOB: 3/18/96

Sex: F

ID: 4785

39

8/23/00

Req. #: A00553:1

count: 463462

Queen Elisabeth

4785

sound dead