Full Application

Title of Proposed Ancillary Study Effect of modifying dietary carbohydrate intake on lipoprotein subclass distribution and particle number in the Framingham State Food Study.

Principal Investigator for Ancillary Study Amy Knapp, PhD and Ann Johnson, PhD, MBA, RD

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Abstract Low density lipoprotein (LDL) subfractions containing a higher proportion of small dense LDL are associated with higher risk for coronary artery disease and is a characteristic of individuals diagnosed with the Metabolic Syndrome and insulin resistance. Diet composition affects lipoprotein particle distribution, in particular, the distribution of large and small LDL particles. Evidence in the literature suggests that a very low carbohydrate diet reduces the number of small LDL particles and favors a greater distribution of large LDL particles. The purpose of this study is to investigate whether isocaloric diets varying in carbohydrate composition (low, med, high) will affect lipoprotein particle distribution and particle number (especially LDL) independent of weight-loss. We hypothesize that due to changes in lipoprotein metabolism, VLDL, LDL and HDL particle size distribution and particle number will be different at the end of the weight loss and maintenance phases of the $(FS)^2$ study.

PROPOSED BUDGET

Personnel		Time/Effort		$ Amount Requested		
Name	Project position title	%	Hours per week	Salary	Fringe benefits	Salary + Fringe
			Subtotal of personnel costs			

Supplies (itemize, expand any boxes as needed)	**Subtotal=**
Patient care costs (itemize) none	**Subtotal=**
Other expenses (itemize) NMR Samples $55/sample x 315 samples (3 time points x 3 treatment groups)	**Subtotal**=$17,325

Total Budget=$17,397

BIOGRAPHICAL SKETCH

Provide the following information for the Principal Investigator. DO NOT EXCEED FOUR PAGES

NAME	POSITION TITLE
Amy Knapp	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of California, Davis, CA	B.S.	06/97	Biology/Physiology
San Diego State University, San Diego, CA (2 years coursework in graduate exercise physiology)	No degree	09/00-06/02	
University of California, San Diego, CA	PhD	09/09	Biomedical Sciences/Physiology
University of California, San Diego	Postdoc	09/09-01/10	Muscle Physiology

A. Personal Statement

Briefly describe why your experience and qualifications make you particularly well-suited for your role as Principal Investigator on the proposed ancillary project. In the section, if appropriate, you also may explain how your expertise might contribute to the parent project.

As a graduate student in exercise physiology and a doctoral candidate in biomedical sciences, I worked on human studies at the University of California San Diego. Through different studies, I was involved with blood collection and running assays, organization and analysis of data and design of a method to test diffusing capacity in humans during exercise.

I have a personal and academic interest in the effects of diet on disease. I teach physiology to the nutrition students at FSU and have incorporated this type of research into my course. I am also very interested in involving some of our Biology students in this project. They complete an independent research project as a requirement for the major and motivated students would benefit from this opportunity. My hope is that they would also be able to help with some of the data collection for the ancillary study. While my past research is not in nutrition and lipid metabolism specifically, I am resourceful, motivated and excited to make this project work within the $(FS)^2$ study.

B. Positions and Honors

List in chronological order previous positions, concluding with the present position. List any honors.

1/2011 – 5/2011	Adjunct Professor	Palomar Community College, CA
09/2011- 07/2013	Assistant Professor	Regis College, MA
09/2013 – present	Assistant Professor	Framingham State University, MA

C. Selected Peer-reviewed Publications

Limit the list of selected peer-reviewed publications, or manuscripts in press, to no more than 15. Do not include manuscripts submitted or in preparation. The individual may choose to include selected publications based on recency, importance to the field, and/or relevance to the proposed ancillary study or parent project.

Philp A, Chen A, Lan D, Meyer GA, Murphy AN, **Knapp AE**, Olfert IM, McCurdy CE, Marcotte GR, Hogan MC, Baar K, Schenk S. Sirtuin 1 (SIRT1) deacetylase activity is not required for mitochondrial biogenesis or peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) deacetylation following endurance exercise. J Biol Chem. 2011 Sep 2;286(35):30561-70.

Stary, C.M., B.J. Walsh, **A.E. Knapp**, D. Brafman, and M.C. Hogan. Elevation in heat shock protein 72 mRNA following contractions in isolated single skeletal muscle fibers. Am J Physiol Regul Integr Comp Physiol. 2008 Aug;295(2):R642-8.

Breen, E., K. Tang, M. Olfert, **A. Knapp**, and P.D. Wagner P. Skeletal muscle capillarity during hypoxia: VEGF and its activation. High Alt Med Biol. 2008 Summer;9(2):158-66.

Olfert, M., J. Balouch, A. Kleinsasser, **A. Knapp**, H. Wagner, P.D. Wagner, and S.R. Hopkins. Does gender affect human pulmonary gas exchange during exercise? *J Physiol*. Volume 557, Number 2, 529-541, June 1, 2004.

D. Research Support

List selected ongoing and completed research projects for the past five years. Begin with the projects that are most relevant to the research proposed in the application. Briefly indicate the overall goals of the projects and your responsibilities.

DISCLOSURES

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Please report relationships that were present during the 36 months prior to submission of your full application.

Section 1. Work Under Consideration for Funding

Do you anticipate receiving receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the proposed work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? ☐ Yes ☒ No

Section 2. Relevant Financial Activities Outside of the Work Under Consideration for Funding

Do you have financial relationships (regardless of amount of compensation) with entities such as a government agency, foundation, commercial sponsor, or academic institution (other than Framingham State University)?

The relationship may be in the form of ***1)*** a grant, ***2)*** personal fees (monies paid to you for services rendered, generally honoraria, royalties, or fees for consulting, lectures, speakers bureaus, expert testimony, employment, or other affiliations), ***3)*** non-financial support (examples include drugs/equipment supplied by the entity, travel paid by the entity, writing assistance, or administrative support), or ***4)*** other.

Are there any relevant conflicts of interest? ☐ Yes ☒ No If yes, please list below.

Name of Entity	Grant?	Personal Fees?	Non-financial Support?	Other?	Comments
	☐	☐	☐	☐	
	☐	☐	☐	☐	
	☐	☐	☐	☐	
	☐	☐	☐	☐	

Section 3. Intellectual Property – Patents and Copyrights

Do you have any patents (planned, pending or issued) broadly relevant to the proposed work? ☐ Yes x No

Section 4. Relationships Not Covered Above

Are there other relationships or activities that could be perceive to influence, or that give the appearance of potentially influencing, how you conduct the proposed ancillary study?

☐ Yes, the following relationships/conditions/circumstances are present (explain below):

☒ No other relationships/conditions/circumstances that present a potential conflict of interest

RESEARCH PLAN

Study Aims and Hypotheses

Specific Aim 1: To evaluate the effects of weight loss on lipoprotein particle size distribution and number.
Hypothesis: Due to changes in lipoprotein metabolism, VLDL, LDL and HDL particle size distribution and particle number will be different at the end of the weight loss phase of the study.

Specific Aim 2: To evaluate the effects of 3 diets varying widely in carbohydrate to fat ratio (high carb, med carb, low carb) on lipoprotein particle size distribution and particle number during weight-loss maintenance.
Hypothesis: Due to changes in lipoprotein metabolism, VLDL, LDL and HDL particle size distribution and particle number will be different between the 3 diet interventions.

Outcomes VLDL, LDL and HDL particle size distribution and particle number will be determined by nuclear magnetic resonance spectroscopy (NMR LipoProfile® test by LipoScience, Inc., Raleigh, NC). All secondary outcomes will be measured in the $(FS)^2$ study and include: total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, non-esterified fatty acids, serum ketones/ketoacids, CRP, and lactate.

Significance Results from this study will help identify the contributions of weight-loss and diet composition to the lipid profiles and resulting cardiovascular risk profiles of overweight individuals.

Background

It is well established in the literature that low density lipoprotein (LDL) subfractions containing a higher proportion of small dense LDL is associated with higher risk for coronary artery disease [1] and is a characteristic of individuals diagnosed with the Metabolic Syndrome and insulin resistance [2]. Increasing evidence suggests that LDL particle distribution and particle number are better predictors of cardiovascular disease than the well-accepted measure of LDL cholesterol content (LDL-C) [3, 4].

Different methodologies have been employed to measure lipoprotein subfraction distribution. The gold standard is vertical spin ultracentrifugation, which separates the lipoprotein subclasses based on their densities. This method is not widely used since it is time consuming, expensive and limited in availability [3]. Both nuclear magnetic resonance (NMR) spectroscopy and gradient gel electrophoresis have been used to show significant correlations between CVD risk and increased numbers of small LDL particles [4]. However, Arsenault and colleagues found slightly better correlations between CVD risk and small LDL and HDL particle size using NMR spectroscopy compared to gradient gel electrophoresis [5].

The proportion of carbohydrates in a diet affects the lipoprotein subfraction profile. High carbohydrate consumption leads to an increase in triglyceride synthesis and consequently an increase in large VLDL particles and small LDL and HDL particles. This is considered a pro-atherogenic phenotype. Many studies have found decreased proportion of small, dense LDL with carbohydrate restricted diets (10-12% carbohydrate) in both overweight participants and participants with atherogenic dyslipidemia[6-9]. Similarly, premenopausal women placed on a reduced carbohydrate diet (40% carbohydrate) had a significant reduction in small LDL particle size [10]. On the other hand, studies that have evaluated the effects of diets with higher proportions of carbohydrates (50-65%) have found either increased levels of small LDL particles or no change in LDL subclass distribution [11, 12].

Current research supports the hypothesis that a low carbohydrate diet results in a more favorable lipoprotein particle distribution, however, some of the limitations of the studies described above are that weight-loss occurred during the diet intervention or only one type of diet was analyzed

in the study. The strengths of measuring lipoprotein subfraction distributions in the subjects recruited for the $(FS)^2$ diet study are the ability to asses the effects of weight-loss and diet independently of each other and to assess differences between 3 controlled diets varying in carbohydrate composition.

Study Outcomes and Assessment Methods

Study Outcomes:

Primary study outcomes for the proposed ancillary study are included in the table below:

Parameter	Unit
VLDL & Chylomicron Particles (total)	nmol/L
Large VLDL & Chylomicrons Particles	nmol/L
Medium VLDL Particles	nmol/L
Small VLDL Particles	nmol/L
LDL Particles (total)	nmol/L
IDL Particles	nmol/L
Large LDL Particles	nmol/L
Small LDL Particles (total)	nmol/L
HDL Particles (total)	µmol/L
Large HDL Particles	µmol/L
Medium HDL Particles	µmol/L
Small HDL Particles	µmol/L
VLDL Size	nm
LDL Size	nm
HDL Size	nm

Assessment Methods:

Fasting blood samples will be collected from subjects three times during the course of the study. Initial blood draws will take place during the baseline assessment followed by blood collection at the post-weight loss assessment and the final assessment.

At least 1 ml of blood is required from each subject at each assessment in order to obtain a plasma sample of 500ul. If possible, it would be prudent to collect 2ml/subject/assessment to account for any handling or sampling errors. Samples will be collected in EDTA tubes.

Following specimen collection, samples will be centrifuged immediately at 3000 rpm for 10-15 minutes. Samples may be refrigerated and spun within 4 hours of collection. Plasma will then be transferred to a new, labeled vial for shipment. Fresh samples must be refrigerated and shipped within 4 days. Frozen samples must be kept at -70°C.

Frozen samples will be shipped via overnight courier to Liposcience, Inc., Raleig, N.C. for anyalysis. Lipoprotein particle analysis will be done by Nuclear Magnetic Resonance Spectroscopy (NMR) as previously described [13].

Sample Size Considerations:

Based on published data, a sample size of 35 subjects per diet group is required to detect an effect size of .6 in small LDL particle concentration with a repeated measures ANOVA, assuming a significance level of .05 and 80% power.

Subject Considerations:

This study does not place any further burden on the subjects, since the blood draws will correspond to the scheduled assessment periods for the parent study.
The blood collection also does not compromise subject safety as the amount of blood drawn will not exceed a safe limit for each individual according to his or her body weight.

Personnel Considerations:

If possible, we anticipate that the nursing staff hired for the parent study would be able to take these additional blood draws with minimal to no extra cost to the parent study. Labeling of tubes, preparation of samples after collection and shipment of samples will be done by Dr. Knapp or trained research methods Biology students, which would also require no extra costs. If this is not possible, we will need to hire a data collection assistant to process the samples.

Statistics A paired t test will be used to determine differences in the primary dependent variables from baseline to the post-weight loss time point. A repeated measures ANOVA will be used to determine differences in the primary outcomes between the 3 diet groups. The within-subject factor will be time (post weight loss and final assessment timepoints). Covariates of age and sex may also be examined using a repeated measures ANOVA. Correlations will be made to determine relationships between changes in plasma triglyceride levels and changes in the dependent variables as well as relationships between changes in body weight and changes in the dependent variables. Differences with a $P < .05$ will be considered significant. Data will be presented as mean ± SD.

Limitations One of the biggest limitations to any human diet study is controlling the dietary intake of the subjects. Much of this variability will be eliminated due to meals being prepared for the subjects but there is still a level of control lost if subjects eat foods outside of what is required by the study.

Another limitation of this study is that methods for measuring lipoprotein subclass distribution are not standardized. Therefore, results obtained either through NMR spectroscopy or gradient gel electrophoresis must be analyzed carefully with respect to other studies that have used different assays.

REFERENCES

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12. Varady KA, Lamarche B, Santosa S, Demonty I, Charest A, Jones PJ. Effect of weight loss resulting from a combined low-fat diet/exercise regimen on low-density lipoprotein particle size and distribution in obese women. *Metabolism.* 2006;55(10):1302-1307.
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