Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed 56-character length restrictions, including spaces.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT
Lowering Lp(a) with Ascorbate, Lysine and Proline

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☒ NO ☐ YES
(If "Yes," state number and title)
Number: Title:

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR — **New Investigator** ☐ **No** ☒ **Yes**

3a. NAME *(Last, first, middle)*
McDade, Larry

3b. DEGREE(S)
M.D.(AM), N.D., Ph.D.

3c. POSITION TITLE
President

3d. MAILING ADDRESS *(Street, city, state, zip code)*
3750 W. Main Street
Norman, OK 73072

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
McDade Institute

3f. MAJOR SUBDIVISION
None

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: (405) 447-6448 FAX: (405) 321-8581

E-MAIL ADDRESS:
perfecthealth@parkonmain.net

4. HUMAN SUBJECTS RESEARCH
☐ No
☒ Yes

4a. Research Exempt ☒ No ☐ Yes
If "Yes," Exemption No.

4b. Human Subjects Assurance No.
Pending

4c. NIH-defined Phase III Clinical Trial
☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☒ No ☐ Yes

5a. If "Yes," IACUC approval Date

5b. Animal welfare assurance no

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
04/01/03	03/31/04	$125,000	$125,000	$250,000	$250,000

9. APPLICANT ORGANIZATION
Name Vitamin C Foundation
Address 15915 N. Bend Drive
Houston, Texas 77073

Institutional Profile File Number (if known)

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☒ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
760526361
DUNS NO. *(if available)*

Congressional District Texas 18th Congressional Dist.

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name Owen R. Fonorow
Title Director
Address 2880 Sun Valley Road
Lisle, IL 60532

Tel 630-416-1438 FAX 630-416-1309
E-Mail ORFonorow@aol.com

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name Owen R. Fonorow
Title Director
Address 2880 Sun Valley Road
Lisle, IL 60532

Tel 630-416-1438 FAX 630-416-1309
E-Mail ORFonorow@aol.com

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI/PD NAMED IN 3a.
(In ink. "Per" signature not acceptable.)

DATE
09/27/02

15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13.
(In ink. "Per" signature not acceptable.)

DATE
09/27/02

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

In 1994 Linus Pauling and Matthais Rath were the first scientists to recommend the importance of decreasing high lipoprotein(a) [Lp(a)] levels. The disappearance of angina symptoms after taking vitamin C and lysine was documented in a case report in 1991. Pauling reasoned that since the lysine binding site was an important factor in the molecular function of Lp(a), adequate doses of lysine would compete for the lysine binding site and thus prevent accumulation of Lp(a) in the blood vessel wall. At the same time, vitamin C would strengthen the collagen matrix of the injured blood vessel wall. No randomized double blind studies were ever done to scientifically prove this to be the case in humans. However, deposition of Lp(a) has been shown to accumulate at sites of blood vessel stress and injury.

Lp(a) is a highly polymorphic particle and a structural analogue of low density lipoprotein (LDL). The presence of plasminogen-like lysine binding sites in apo(a) led researchers to think that fibrin binding plays an important role in Lp(a)'s vascular accumulation. Plasminogen is the precursor of plasmin, which is a fibrinolytic enzyme. The homology of Lp(a) to plasminogen allows it to bind to fibrin and inhibit plasminogen binding and plasmin generation. Furthermore, insufficient activation of transforming growth factor-beta (TGF-beta) due to low plasmin activity may result in migration and proliferation of smooth muscle cells into the intima, resulting in intimal hyperplasia. The apo(a) isoform with the highest affinity for fibrin could be predictive of vascular disease. Results in transgenic mice indicate a correlation between lysine binding properties of Lp(a) and vascular accumulation, supporting the postulated role of apo(a) lysine binding sites and the atherogenic characteristic of Lp(a).

Therefore, additional controlled studies of subjects with elevated Lp(a) levels treated with various inexpensive and nontoxic interventions such as with 3 or more grams of vitamin C and 3 or more grams of lysine per day need to be done. This pilot study will measure Lp(a) with the Atherotech VAP test to assess for the changes occurring after interventional treatment. Future studies are required to measure isoforms of apo(a), lysine binding site activity, fibrinogen levels, and transforming growth factor beta activity.

PERFORMANCE SITE(S) *(organization, city, state)*

Vitamin C Foundation, Houston, Texas

Alternative Health Care Associates, L.L.C., Silver Springs, Florida

McDade Institute, Norman, Oklahoma

Tower Laboratories Corporation, Las Vegas, Nevada

Atherotech, Birmingham, Alabama

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name	Organization	Role on Project
McDade, Larry	McDade Institute	Principal Investigator
Badanek, Michael J.	Alternative Health Care Associates	Co-investigator
Dalessandri, Kathie M.	Univ. of California at Berkeley	Consultant
Fonorow, Owen R.	Vitamin C Foundation	Program Director
Levin, Warren M.	Private Practice	Consultant
Podlaski, John A.	Private Practice	Co-investigator
Ruminer, Tina	McDade Institute	Co-investigator

Disclosure Permission Statement. Applicable to SBIR/STTR Only. See instructions. ☐ **Yes** ☐ **No**

The name of the principal investigator/program director must be provided at the top of each printed page and each continuation page.

RESEARCH GRANT

TABLE OF CONTENTS

(Items A-D: not to exceed 25 pages)*

** SBIR/STTR Phase I: Items A-D limited to 15 pages.*

Appendix *(Five collated sets. No page numbering necessary for Appendix.)*

Check if Appendix is Included ☐

Appendices NOT PERMITTED for Phase I SBIR/STTR unless specifically solicited.

Number of publications and manuscripts accepted for publication *(not to exceed 10)* _____

Other items (list):

BUDGET JUSTIFICATION PAGE MODULAR RESEARCH GRANT APPLICATION				
Initial Budget Period	**Second Year of Support**	**Third Year of Support**	**Fourth Year of Support**	**Fifth Year of Support**
$125,000	$125,000	$0	$0	$0
Total Direct Costs Requested for Entire Project Period				$250,000

Personnel

Larry McDade, M.D., Ph.D., N.D., Principal Investigator, (15% effort) and Tina Ruminer, M.D., Ph.D., N.D., Co-investigator, (15% effort) operating remotely from the McDade institute will be responsible for scientific oversight and data analysis. Dr. McDade will select subjects from the initial screening. The McDade Institute will collect and monitor the raw data from Florida and Atherotech.

Michael J. Badanek, D.C., Co-investigator, (40% effort) and John A. Podlaski, D.C., Co-investigator, (20% effort) will be responsible for data collection. These physicians will be the on-site medical team. They will first conduct the initial screening. After the McDade Institute selects 40 subjects, Drs. Badanek and Podlaski will collect weekly CardioVision measurements. Blood will be drawn, labeled, spun and sent to Atherotech every two weeks. These physicians will dispense and monitor the Tower ASCORSINE (vitamin C, lysine and proline) product after the baseline period.

Owen R. Fonorow, M.S., M.B.A., Program Director, (30% effort) Mike TIll, Sr., M.B.A., (15%) Controller, and Sally Jewell (10% effort) auditor of the Vitamin C Foundation will be responsible for project management. The Foundation will interface with the Atherotech lipid laboratory, the product supplier Tower Laboratories, the Linus Pauling Institute and other consultants.

Consortium

None

Fee (SBIR/STTR Only)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Owen R. Fonorow	Program Director

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
United States Air Force Academy, CO	B.S.	1972-1976	Computer Science
University of Wyoming, Cheyenne, WY	M.B.A.	1977-1979	Computer Option
Universiity of Arizona, Tucson, AZ	M.S.	1981-1984	Computer Science

Mr. Fonorow is President and Co-founder of the Vitamin C Foundation, having become interested in orthomolecular medicine from both the books of and interactions with LInus Pauling.

After graduating from the U. S. Air Force Academy in 1976, he was commissioned as a 2nd Lt. regular USAF. Captain Fonorow, as a SAC Minuteman Missile Combat Crew Commander stationed at F. E. Warren AFB, Cheyenne, WY, became the Base Commander's Executive Officer.

After leaving the Air Force with an honorable discharge in 1981, Owen studied computer programming languages at the University of Arizona, graduating with a Master's degree in Computer Science.

In 1983, after reading Nobelist Linus Pauling's book The Common Cold and the Flu, he began taking 3 g of vitamin C daily. Soon thereafter multiple recurring infections and childhood illnesses were "cured" never to return.

He joined AT&T Bell Laboratories as a Member of the Technical Staff in 1984 and held various technical computer and telephony positions at AT&T and Lucent. He was awarded a patent for an automated testing system and was a speaker at conferences and author of technical papers.

In 1986, after reading Pauling's book How to Live Longer and Feel Better, and Dr. Robert Cathcart's famous Titrating Vitamin C to Bowel Tolerance paper (provided by Pauling), he increased his daily vitamin C intake to 18,000 mg (18 g), matching Pauling's intake. At this point, other conditions cleared up. Specifically diagnosed gout, allergies, and especially hay-fever were "cured" never to return. Fascinated, he began private research into vitamin C and the other nutrients.

After recent retirement from Lucent's Bell Labs, he now teaches the UNIX system at a local technical college and is pursuing a Ph. D. in Natural Health and Nutritional approaches to disease. Mr. Fonorow has designed and maintains four large web sites devoted to various aspects of health and nutrition, including the Vitamin C Foundation's web site.

He has written more than a dozen articles and papers published in assorted Alternative Medical journals including the Townsend Letter, American Naturopathic Medical Association Monitor, Florida ECO Report, International Council for Health Freedom Newsletter and Media By-Pass.

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Atherotech
400 Vestavia Parkway
Suite 300
Birmingham, AL 35216 (800)719-9807 Contact: Richard B. Lanman MD, (408) 390-9357

Clinical:

Alternative Health Care Associates L.L.C. (100%)
3391 East Silver Springs Blvd
Suite B
Ocala, Florida 34470
Silver Springs, FL 34489 Badanek: (352) 622-1151 Podlawski: (352) 867-1015

Animal:

None

Computer:

The Vitamin C Foundation, 2880 Sun Valley Road, Lisle, IL, 60532, Owen Fonorow (630)416-1438
The Vitamin C Foundation, 15915 North Bend Road, Houston, TX, 77073, Mike Till (271)334-3634

Office:

The McDade Institute, 3750 W. Main Street, Norman, OK 73072, (405) 447-6448

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Laptop and Desktop Computers (Lisle, IL, and Houston, TX)
Centrifuge (Silver Springs, FL)

R21 GRANT PROPOSAL

Submitted to the National Center for Complementary and Alternative Medicine
National Institutes of Health

Lowering Lp(a) with Ascorbate, Lysine and Proline in Humans
A Pilot Study
September 27, 2002
The VITAMIN C FOUNDATION

Research Plan

The Foundation was approached by the Investigators after they observed Lp(a) reductions in their private practices. Approximately 100 elderly individuals in Silver Springs, Florida, members of a local church, have volunteered to participate in this study. Forty with elevated Lp(a) and not currently supplementing with lysine and proline will be screened and enrolled. An existing commercial vitamin C/lysine/proline drink mix will be used to improve compliance and reduce the chance for error.

a. Specific Aims

To obtain objective clinical data on the value of Linus Pauling's suggested intervention of 6 g ascorbic acid (vitamin C) and 6 g lysine, plus 500 mg proline, to test the following hypotheses:

PRIMARY:

This intervention lowers Lp(a) by more than 40% within 6 months.

SECONDARY:

1. This intervention lowers Lp(a) more than 90% within 18 months.

2. This intervention leads to a predictable reduction in accepted cardiovascular risk factors such as LDL-C, homocysteine and hsCRP.

3. This intervention leads to a predictable reduction in Brachial Arterial Stiffness Index (ASI) using the FDA-approved CardioVision screening device for atherosclerosis.

4. This intervention leads to a predictable reduction in angina pectoris (chest pain).

5. There is a correlation between serum Lp(a) and Arterial Stiffness Index (ASI)

Finally, as funds permit and as a secondary control mechanism, the Investigators will compare the accuracy of a new low-cost "home" Lp(a) test coming on the market with the Lp(a) results from the same blood as reported by Atherotech's certified VAP™ test.

b. Background and Significance

Note: Dr. Kathie Dalessandri wrote the following sections on the Past, Present and Future Lp(a) research (i.e., sections b.(1), b.(2) and b.(3)) for the International Atherosclerosis Society (IAS) website: www.athero.org , at the request of Dr. Scott Grundy, M.D. who is the Director for the Center for Human Nutrition at University of Texas Southwestern Medical Center in Dallas.

b.(1) Past Lipoprotein(a) Research

In 1994, Linus Pauling and Matthias Rath were the first scientists to recommend the importance of decreasing high lipoprotein(a) [Lp(a)] levels. They believed that oral intake of ascorbic acid (vitamin C) and lysine would accomplish this goal [1, 2]. The disappearance of angina symptoms after taking vitamin C and lysine was documented in a case report in 1991 [3]. Pauling reasoned that since the lysine binding site was an important factor in the molecular function of Lp(a), adequate doses of lysine would compete for the lysine binding site and thus prevent the accumulation of Lp(a) in the blood vessel wall [1, 2]. At the same time, vitamin C would strengthen the collagen matrix of the injured blood vessel wall [1, 2]. No randomized double-blind studies were ever conducted to scientifically prove this to be the case in humans. However, deposition of Lp(a) has been shown to accumulate at sites of blood vessel stress and injury [4].

Lp(a) is a highly polymorphic particle and a structural analogue of low density lipoprotein (LDL), which contains two distinct proteins: apolipoprotein(a) [apo(a)] that is covalently linked to apolipoprotein B-100. The presence of plasminogen-like lysine binding sites in apo(a) led researchers to think that fibrin binding plays an important role in Lp(a)'s vascular accumulation. Plasminogen is the precursor of plasmin, which is a fibrinolytic enzyme. The homology of Lp(a) to plasminogen allows it to bind to fibrin and inhibit plasminogen binding and plasmin generation. Furthermore, insufficient activation of transforming growth factor-beta (TGF-beta) due to low plasmin activity may result in migration and proliferation of smooth muscle cells into the intima, resulting in intimal hyperplasia. The apo(a) isoform with the highest affinity for fibrin could be predictive of vascular disease [5]. Results in transgenic mice indicate a correlation between the lysine binding properties of Lp(a) and vascular accumulation, supporting the postulated role of apo(a) lysine binding sites and the atherogenic characteristic of Lp(a) [6, 7].

b.(2) Present Lipoprotein(a) Research

Elevated Lp(a) is considered to be a major independent risk factor for coronary artery disease [8]. Thirty-seven percent of the U. S. population who are at high risk for coronary artery disease have elevated Lp(a) levels above 25 mg/dl [8]. British researchers who analyzed the findings from 27 studies found that those patients with high levels of Lp(a) had a 70% higher risk of heart disease [9]. The risk for coronary artery disease also appears to be synergistic in some individuals with other associated lipid abnormalities such as elevated LDL, elevated apolipoprotein B, low HDL, elevated fibrinogen, and elevated C-reactive protein and those with small apolipoprotein(a) size and high lysine binding site activity [10-15]. Individuals who are more susceptible to morning myocardial infarction rates have been found to have high lipoprotein(a) levels [16].

Measurement of Lp(a) levels has not yet been standardized, but a grant from the National Heart, Lung and Blood Institute, National Institutes of Health, has awarded a contract for the standardization of these measurements so that future research from a variety of sites can be better compared [10].

Testing of Lp(a), apo(a), and lysine binding sites as they interrelate with cardiac risk has been documented primarily in highly refined lipid laboratories studying Lp(a) and its relationship to atherosclerosis [10, 12, 15]. It appears that there is an association between small isoforms of apo(a), high lysine binding site activity, and high Lp(a) concentrations and disease in humans [15].

b.(3) Future Lipoprotein(a) Research

The new field of nutragenomics will have an influence on future Lp(a) research. Nutragenomics is defined as the effect of nutrition and nutritional supplements on certain polymorphisms. Functional foods and supplements could have very specific health benefits based on an individual's genotype. Lp(a) has at least 34 polymorphs which could be influenced by various gene-environment interactions [17]. There has been a recent case report published in the Archives of Internal Medicine of a remarkable decrease of 48% in Lp(a) levels after a six-month trial of 3 grams of ascorbic acid and 3 grams of L-lysine monochloride per day, with no side effects [18]. The results were documented by the highly reliable Lawrence Berkeley National Laboratory/Berkeley HeartLab Department Technology Transfer program [18]. Also published recently is a report in Atherosclerosis of multiple methods for reduction of Lp(a), which include use of niacin, vitamin C and lysine, estrogen and progestin, tamoxifen, L-carnitine, and diet [19]. One randomized, placebo-controlled study and one nonrandomized, nonplacebo-controlled study in subjects with diabetes have demonstrated that niacin can lower Lp(a) levels [20,21].

Gene environment interactions are important in assessing the impact of gene variants (polymorphisms) [22, 23]. Therefore, additional randomized, placebo-controlled studies of subjects with elevated Lp(a) levels treated with various inexpensive and nontoxic interventions such as 3 grams of vitamin C and 3 grams of lysine per day need to be performed. Levels of Lp(a) as well as isoforms of apo(a), lysine binding site activity, fibrinogen levels, and transforming growth factor beta activity could be measured in lipid research laboratories to assess changes occurring after interventional treatment. Once this is done and the results analyzed, a larger clinical trial could take place to assess the effect of lowering Lp(a) and small apo(a) levels on the morbidity and mortality of cardiac events.

c. Preliminary Studies

Pauling and Rath, testing their unified theory, performed experiments with hypoascorbemic guinea pigs [24]. Repeating the early work of Willis [25], they showed that Lp(a)/apo(a) becomes elevated in these animals without endogenous vitamin C when deprived (compared to controls). Lp(a) was discovered in the atherosclerotic lesions. As per Willis, the controls were without atherosclerotic lesions.

The phenomenon of Lp(a) lowering more than 40% has been reported by alternative/complementary physicians experienced with Pauling's high-dose vitamin C/lysine protocol since 1995. This trial will be the first to test the hypothesis that once elevated, hypoascorbemia-induced Lp(a) can be predictably lowered using a low-cost intervention.

d. Research Design and Methods

The Investigators approached the Vitamin C Foundation citing the need for this study. They reported significant reductions in Lp(a) utilizing the recommended Pauling protocol in their private practices of alternative medicine. Approximately 100 elderly members of a local church in Silver Springs, Florida have volunteered to participate in this 6 to 18-month pilot study. Forty with elevated Lp(a) and not currently supplementing lysine and proline will be screened and enrolled.

The Investigators are licensed in the state of Florida. They can draw blood, spin, and label samples for shipment to the lipid laboratory in Alabama. One local Investigator is a Board Certified internist, and both Investigators have close ties with several local cardiologists.

For ethical reasons, the volunteers will not be deprived of vitamin C. Rather than using a placebo group, this study design will gather all measurements on all subjects during an introductory period of not less than two months. These initial measurements, without intervention, will provide the statistical baseline.

The study will utilize the advanced lipid test, the VAP™ (Vertical Auto Profile) Cholesterol Test developed by Atherotech, Inc. of Birmingham, Alabama. The study will gather additional data, including Brachial Arterial Stiffness Index (ASI) data using the FDA approved CardioVision atherosclerosis screening unit.

An existing commercial vitamin C/lysine/proline drink mix, familiar to all Investigators, will be used to improve compliance and reduce chances for error. The product, ASCORSINE (HeartTechnology brand) from Tower Orthomolecular Laboratories of Las Vegas, Nevada, includes all of the basic Linus Pauling recommendations as a drink mix. This method of administration reduces the chances of subjects taking either the wrong pills or the wrong number of pills. The non-placebo design and the product's pleasant taste (Stevia) promotes compliance.

Tower reports that it has received many anecdotal reports of benefit attributed to ASCORSINE since its inception in 1996, especially concerning the lowering of serum Lp(a) by more than 40%.

The Investigators will periodically monitor serum ascorbate to verify compliance with the protocol.

d.(1) Protocol

Step One. The local Investigators will screen the volunteers for elevated Lp(a), above 25 mg/dl standard (15 mg/dl VAP). The Principal Investigator will select 40 subjects with the highest serum Lp(a) to participate. The screening process will include entrance criteria such as high Arterial Stiffness (ASI), complaints of angina pectoris, and not supplementing lysine/proline. Those with evidence of coronary disease will be favored.

Step Two. All 40 study participants will be monitored bi-weekly for two months without intervention to provide the statistical baseline.

Step Three. The study subjects will be supplied with Tower ASCORSINE and will be monitored bi-weekly for a period of no less than six months and no longer than 18 months. After six months, VAP blood testing will be reduced to one per month. The study will terminate after two successive months or 18 months without a statistically significant change in Lp(a), whichever is sooner.

The Investigators will monitor compliance and measure Lp(a), as well as all cholesterol risk factors, including LDL-C and LDL, HDL, and VLDL density measurements. In addition, several high-value indicators of heart and cardiovascular health will be monitored. These indicators will include but may not be limited to angina pectoris pain, brachial Arterial Stiffness Index (ASI), weight, blood pressure, homocysteine, hsCRP, and other blood and physical examinations which the physicians and consultants deem appropriate.

d.(2) Atherotech's VAP Test

The VAP Test provides a detailed lipid profile based on the cholesterol content of all lipoprotein particles in the blood. The test requires five drops of blood, which may be obtained via finger-stick or blood draw. The sample is sent to Atherotech's CLIA (Clinical Laboratory Improvement Act)-approved, CDC-certified lipid laboratory in Birmingham for processing.

The results are obtained by means of density gradient ultracentrifugation, a highly accurate process that separates lighter portions of lipoprotein from heavier portions by means of centrifugal force. Atherotech participates in, and has its accuracy confirmed by, two prominent lipid standardization programs: the New York State Department of Health and the Northwest Lipid Research Laboratories.

The VAP test is emerging as the new standard of care for cholesterol risk assessment because it meets the New Cholesterol Education Program (NCEP) guidelines by directly measuring all cholesterol risk factors, including LDL-C and Lp(a), and providing LDL, HDL and VLDL density measurements. Although LDL-C remains the primary focus of risk reduction strategies, the newly recognized Secondary Targets of Therapy identified by the NCEP guidelines include LDL-C particle density, Lp(a), and TG-rich lipoproteins (IDL, VLDL, and small, dense VLDL).

Of the lipid parameters which the VAP Test addresses, research shows that small, dense LDL (Pattern B) is the most important but that next in importance is Lp(a) because of its well-documented role as a major cardiovascular risk factor. In fact, elevated Lp(a) is associated with a 70% increase in heart attack risk, making it one of the four primary predictors of risk along with LDL, HDL and small, dense LDL.

Researchers at Duke University are using the VAP Test in a landmark study monitoring the impact and benefits of exercise on cholesterol levels. Additionally, researchers at the Oregon Research Institute have selected the test for use in a National Heart, Lung and Blood Institute-sponsored study evaluating the benefits of lifestyle changes for women at risk for heart disease. The VAP Test has also been chosen by Merck for studies evaluating the efficacy of its cholesterol-lowering drugs.

For more information concerning the VAP Cholesterol Test, please call 1-877-901-8511 or visit Atherotech's web site at www.atherotech.com.

d.(3) CardioVision/Arterial Stiffness Index (ASI) Measurements

CardioVision is an extremely sensitive, non-invasive device for screening arterial elasticity or arterial stiffness. When used as a screening device, it indicates whether further testing (evaluation) may be required because of hypertension or dysfunctional arteries.

Arterial Stiffness Index (ASI) is a dynamic physiologic quantitative value. According to product literature, the unit is FDA approved for screening atherosclerosis. A high to very high ASI value indicates a high to very high risk of coronary artery disease.

The unit does not measure cardiac output but the averaging technique is similar. Since the variation in values obtained can change so rapidly, the test is run three to five times and then averaged. If any value is more than 50% above or below average, it is rejected and the test is run again and averaged. The procedure is repeated until all results fall within the accepted range, and then the average is output (the Stiffness Index).

The unit measures blood pressure (hundreds of measurements per second) and is integrated with a personal computer. The software can be used as a database to record patient data. Easy-to-read graphical representations of arterial stiffness over time are produced.

CardioVision has been chosen for this investigation to help with initial screening and also because one woman in the Chappell study [26] who did not respond to EDTA chelation (subject 129-98), did respond and lower her ASI (from over 200 ASI) to below 60 ASI in 30 days with Tower ASCORSINE (Contact: Dr. Robert Angus). The unit produces its own results and is cost effective: Investigators can measure ASI weekly at no extra cost after the initial purchase (around $5,000).

d(4) Tower ASCORSINE (HeartTechnology) Drink Mix

Tower Laboratories Corporation, d.b.a. Tower Orthomolecular Labs, 3305 W. Spring Mountain Road, Las Vegas, Nevada 89102, (702) 876-5805, has been in existence manufacturing and marketing its ASCORSINE product to the general public since 1996. Tower's formulas are based on both long-time and the most recent recommendations of Linus Pauling.

ASCORSINE Product Description (amounts per serving, 2 servings daily)

Vitamin C (ascorbic acid)	3,000 mg
L-lysine	2,800 mg
L-proline	250 mg
Vitamin A (palmitate)	8,500 IU
Vitamin B-2 (riboflavin)	3 mg
Vitamin E (dl-alpha tocopherol)	422 IU
L-carnitine	250 mg
Coenzyme Q-10	10 mg
Stevia Rebaudiana extract	55 mg

The ASCORSINE/HeartTechnology brand products are mixed and bottled at Nutri Pharmaceuticals, Inc., 3566 Polaris Avenue, Las Vegas, Nevada 89103, (702) 871-6300, npi.e-nutri.net, a licensed and State inspected facility in Las Vegas, Nevada. NPI uses the newest laboratory instrumentation and equipment that ensures the highest quality of production. Strict control measures are performed. The facility is equipped to handle powder blending, granulation, tableting, coating and encapsulation, and NPI offers a full line of packaging services. NPI also offers an in-house laboratory to conduct a majority of testing using their Research Scientist and Chief Chemist with over 30 years of experience.

Tower/NPI will provide a detailed chemical analysis of the ASCORSINE product with the initial delivery to the study site.

e. Human Subjects

e.(1) RISKS TO THE SUBJECTS

There are no known toxicities from the vitamin and amino acid intervention to be investigated. Dr. Badanek has found more than one hundred volunteers willing to participate in this study from his local church. They will be screened, and 40 subjects with elevated Lp(a), a recognized cardiovascular risk factor, will be selected, regardless of age, sex, race or religion. Those selected will be examined by the Investigators in their medical offices weekly during the first six months of the study and bi-weekly thereafter. Blood will be drawn bi-weekly, i.e. during every other visit.

e.(2) ADEQUACY OF PROTECTION AGAINST RISKS

Subject safety is the primary consideration of the study design. The study minimizes potential risks by a) using an existing commercial nutritional product that the Investigators currently use in their practices and that has been widely sold for the past seven years; b) by not utilizing a placebo and thereby depriving some subjects of possible benefit; c) by appointing experienced physicians as Investigators, and e) by monitoring the subjects' general health weekly. In particular, there are no known risks (i.e. no radiation) from the planned measurement devices, e.g., the CardioVision unit is non-invasive. The Investigators will work closely with local cardiologists to create a fast-response team should the need for one arise.

e.(3) POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

The subjects will have elevated Lp(a) and previous research shows they can expect at least an 18% to 30% drop in this important cardiovascular risk factor within six months. Weekly visit's to the Investigators private practices at "no charge" is one reason why 100 have already volunteered. Free blood testing is another benefit, as is continual ASI monitoring for possible coronary disease.

e.(4) IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

According to the American Heart Association, Cardiovascular Disease (CVD) contributed to more than a third of global deaths in 1999. The numbers are staggering: 61,800,000 Americans have one or more types of cardiovascular disease according to current estimates. Of these, 29,700,000 are male and 32,100,000 are female. 24,750,000 are estimated to be age 65 and older.

- High blood pressure - 50,000,000.
- Coronary heart disease - 12,600,000.
- Myocardial infarction - 7,500,000.
- Angina pectoris - 6,400,000.
- Stroke - 4,600,000.
- Congenital cardiovascular defects - 1,000,000.
- Congestive heart failure - 4,790,000.
- 1 in 5 males and females has some form of cardiovascular disease

It has been known since 2000 that individuals with elevated Lp(a) are 70% more likely to suffer an early heart attack or stroke. This pilot study may provide important data on a low-cost intervention for lowering Lp(a) and may provide additional support for the Linus Pauling/Matthias Rath theory that elevated Lp(a) is acting as a surrogate for chronically deficient vitamin C.

References

1. Rath M, Pauling L. Lipoprotein(a) is a surrogate for ascorbate. Proc Natl Acad Sci 1990; 87:6204-7.
2. Pauling L, Rath M. Solution to the puzzle of human cardiovascular disease: Its primary cause is ascorbate deficiency leading to the deposition of lipoprotein(a) and fibrinogen/fibrin in the vascular wall.
J Orthomolecular Med 1991; 6:125-34.
3. Pauling L. case report: Lysine/ascorbate-related amelioration of angina pectoris. J Orthomolecular Med 1991; 6:144-46.
4. Niendorf A, Rath M, Wolf K, et al. Morphological detection and quantification of lipoprotein(a) deposition in atheromatous lesions of human aorta and coronary arteries. Virchow's Archiv A Pathol Anat 1990; 417:105-11.
5. Angles-Cano E. Structural basis for the pathophysiology of lipoprotein(a) in the athero-thrombotic process. Braz J Med Biol Res 1997; 30(11): 1271-80.
6. Hughes SD, Xing JL, Ighani S, Verstuyft J, et al. Lipoprotein(a) vascular accumulation in mice. J Clin Invest 1997; 100(6): 1493-1500.
7. Boonmark NW, Xing JL, Yang ZJ, et al. Modification of apolipoprotein(a) lysine binding site reduces atherosclerosis in transgenic mice. J Clin Invest 1997; 100(3): 558-64.
8. Superko HR, Hecht HS. Metabolic disorders contribute to subclinical coronary atherosclerosis in patients with coronary calcification. Am J Cardiol 2001; 88(3): 260-64.
9. Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 2000:102:1082-85.
10. Marcovina SM, Koschinsky L. Lipoprotein(a) concentration and apolipoprotein(a) size a synergistic role in advanced atherosclerosis? Circulation 1999; 100:1151-53.
11. Dangas G, Mehran R, Harpel PC, et al. Lipoprotein(a) and inflammation in human coronary atheroma: Association with the severity of clinical presentation. J Am Coll Cardiol. 1998; 31:519-25.
12. Ducas J, Silversides C, Dembinski, et al. Apolipoprotein(a) phenotypes predict the severity of coronary artery stenosis. Med Clin Exp 2002; 25(3):74-81.
13. Cantin B, Despres J B, Lamarche B, Moorjani S, et al. Association of fibrinogen and lipoprotein(a) as a coronary heart disease risk factor in men (the Quebec Cardiovascular Study). Am J Cardiol 2002; 89:663-66.
14. Kronenberg F, Kronenberg M F, Keichl S, et al. Role of lipoprotein(a) phenotype in atherogenesis. Prospective results from the Bruneck Study. Circulation 1999; 100:1154-60.
15. Simo J M, Joven J, Vilella E, et al. Impact of apolipoprotein(a) isoform size heterogeneity on the lysine binding function of lipoprotein(a) in early onset coronary artery disease. Thromb Haemost 2001; 85(3): 412-17.
16. Fujino T, Katou J, Fujita M, et al. Relationship between serum lipoprotein(a) level and thrombin generation to the circadian variation in onset of acute myocardial infarction. Atherosclerosis 2001; 155:171-78.
17. Sniderman A D. Lipoprotein(a) and atherogenesis: A puzzle inside a riddle within an enigma. Clin Invest Med 2002; 25(3): 89-91.
18. Dalessandri, K. Reduction of lipoprotein(a) in postmenopausal women. Arch Int Med 2001; 161(5): 772-73.
19. Dalessandri, K. Multiple methods for reduction of lipoprotein(a). Atherosclerosis 2002; 163:409-10.
20. Pan J, Lin M, Kesala R L, Van J, Charle M A. Niacin treatment of the atherogenic lipid profile and Lp(a) in diabetes. Diabetes Obes Metab 2002; 4(4): 255-61.

21. Grundy S M, Vega G L, McGovern M E, et al. Efficacy, safety and tolerability of once-daily niacin for the treatment of dyslipidemia associated with type 2 diabetes: Results of the assessment of diabetes control and evaluation of the efficacy of niaspan trial. Arch
Intern Med 2002; 162(14): 1568-76.
22. Simo J M, Joven J, Vilella E, et al. Polymorphisms in human apolipoprotein(a) kringle IV-10 and coronary artery disease: Relationship to allele size, plasma lipoprotein(a) concentration and lysine binding site activity. J Mol Med 2001; 79(5-6): 294-99.
23. Becker L, McLeod R S, Marcovina S M, et al. Identification of a critical lysine residue in apolipoprotein B-100 that mediates noncovalent interaction with apolipoprotein(a). J Biol Chem 2001; 276(39): 36155-62.
24. Pauling L, Rath M. Immunological evidence for the accumulation of lipoprotein(a) in the atherosclerotic lesion of the hypoascorbemic guinea pig. Proc Natl Acad Sci USA Dec, 1987; 87:9388-9390.
25. Willis G C. The reversibility of atherosclerosis. Canadian Medical Assoc J. 1957; Vol 77:106-109.
26. Chappell T L, Angus R C, Stahl J P, Evans R. Brachial artery stiffness testing as an outcomes measurement for EDTA-treated patients with vascular disease. Clinical Prac of Alt Med 2000;Vo1 1, No 4:225-228.

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☒ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ SBIR Phase I ☐ SBIR Phase II: SBIR Phase I Grant No. _ _____ ☐ SBIR Fast Track

☐ STTR Phase I ☐ STTR Phase II: STTR Phase I Grant No. _ _____ ☐ STTR Fast Track

☐ REVISION of application number: _____

(This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)

☐ COMPETING CONTINUATION of grant number: _____

(This application is to extend a funded grant beyond its current project period.)

INVENTIONS AND PATENTS *(Competing continuation appl. and Phase II only)*

☐ No ☐ Previously reported

☐ Yes. If "Yes," ☐ Not previously reported

☐ SUPPLEMENT to grant number: _____

(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of principal investigator/program director.

Name of former principal investigator/program director: _____

☐ FOREIGN application or significant foreign component.

1. PROGRAM INCOME ***(See instructions.)***

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***

The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/ certifications are provided in Section III. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

•Human Subjects; •Research Using Human Embryonic Stem Cells• •Research on Transplantation of Human Fetal Tissue •Women and Minority Inclusion Policy •Inclusion of Children Policy• Vertebrate Animals• •Debarment and Suspension; •Drug- Free Workplace *(applicable to new [Type 1] or revised [Type 1] applications only)*; •Lobbying; •Non-Delinquency on Federal Debt; •Research Misconduct; •Civil Rights (Form HHS 441 or HHS 690); •Handicapped Individuals (Form HHS 641 or HHS 690); •Sex Discrimination (Form HHS 639-A or HHS 690); •Age Discrimination (Form HHS 680 or HHS 690); •Recombinant DNA and Human Gene Transfer Research; •Financial Conflict of Interest (except Phase I SBIR/STTR) •STTR ONLY: Certification of Research Institution Participation.

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☐ DHHS Agreement dated: _____ ☒ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with _____ Regional Office.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $		x Rate applied		% = F&A costs	$
b. 02 year	Amount of base $		x Rate applied		% = F&A costs	$
c. 03 year	Amount of base $		x Rate applied		% = F&A costs	$
d. 04 year	Amount of base $		x Rate applied		% = F&A costs	$
e. 05 year	Amount of base $		x Rate applied		% = F&A costs	$
					TOTAL F&A Costs $	

*Check appropriate box(es):

☐ Salary and wages base ☐ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

4. SMOKE-FREE WORKPLACE ☐ Yes ☒ No *(The response to this question has no impact on the review or funding of this application.)*