NCT01847027

UNT Health Science Center
Office for the Protection of Human Subjects
Institutional Review Board
BOARD ACTION

IRB Project #: 2012-162 Date Submitted: INITIAL APPROVAL

Principal Investigator: Meharvan Singh, PhD

Project Title: Exercise and NT-020 (NutraStem): Effects on Stem Cells

Sponsor Protocol #:

Department: Pharmacology and Neuroscience Contact Info: K. Brown x 2056

In accordance with UNT Health Science Center policy on the protection of human subjects, the following action has been taken on the above referenced project. Approval, when given,is **only** for the project as submitted. **No changes** may be implemented without first receiving IRB review and approval.

The Principal Investigator must notify the IRB immediately if any new potential Conflict of Interest arises or if CITI educational training lapses for any of the Key Personnel involved with the study.

☑ Project has received approval through: **December 4, 2013**

☑ Informed consent(s*) approved as submitted on : **January 4, 2013**

You **MUST** use the version (s) attached rather than previously approved versions. In addition, only consent documents which bear the official UNTHSC IRB approval stamp can be used with subjects.

*Including:

☐ Study Protocol dated _____ approved as submitted.

☐ Investigator's Brochure _____ approved as submitted.

☑ Protocol Synopsis approved as submitted on: **January 4, 2013**

☐ Amendment _____ to the protocol approved as submitted.

☐ Progress Report/Continuing Review completed, project has received approval through: _____

☐ Project has been reviewed. In order to receive approval, you must incorporate the attached modifications. You must submit one "tracked changes" version showing the markup and one "clean" copy of the revised protocol synopsis, informed consent, and advertisements to the IRB for review. ***YOU MAY NOT BEGIN YOUR PROJECT UNTIL NOTIFIED BY THE IRB.***

☐ Project is disapproved for the reason(s) outlined (see attached).

☐ Consideration of the project has been **DEFERRED** pending resolution of the issues(s) outlined (see attached).

☐ Completion of project is acknowledged and all required paperwork has been received.

☐ Special Findings/Other

See Page 2

Chairman, Institutional Review Board

January 4, 2013
Date

PI: Meharvan Singh, PhD

IRB Project #: 2012-162

Date: 01/04/2013

SPECIAL FINDINGS:

☐ CHILDREN: The Board found the participation of children to be approvable under Subpart D of the federal regulations. Specifically, the research satisfies the requirements of:

☐ **45 CFR** ☐ **21 CFR**

☐ COGNITIVELY IMPAIRED: The Board found the participation of cognitively impaired subjects to be approvable under federal regulations. Specifically, the research satisfies the requirements of:

☐ **45 CFR 46.111 (b)** ☐ **21CFR 56.111 (b)**

☐ PREGNANT WOMEN: The Board found the participation of pregnant female subjects to be approvable under Subpart B of federal regulations. Specifically, the research satisfies the requirements of: **45 CFR 46.204 (a) - (i)**

☐ FETUSES/NEONATES: The Board found the involvement of fetuses/neonates to be approvable under *Subpart B* of federal regulations. Specifically, the research satisfies the requirements of: **45 CFR**

☐ PRISONERS: The Board found the participation of prisoners to be approvable under *Subpart C* of federal regulations. Specifically, the research satisfies the requirements of: **45 CFR 46.305 (a), (b) and (c)**

☐ OTHER:

OTHER

☐ **Expedited Review Procedures (under 45 CFR 46)**

Project ☐ Approved ☐ Approved for Continuation ☐ Modifications approved **under the provisions of:**

☐ **45 CFR 46.110 (b) (2)** minor changes in previously approved research during the period (of one year or less) for which approval is authorized.

☐ **HIPAA Waiver:** The Board finds this study meets all legal requirements for a Waiver of Individual Authorization under HIPAA pursuant to 45 CFR 164.512 (i) (2) (i)-(v) and approves the request under:

☐ **Informed Consent Waiver:** The Board finds this project qualifies for a _____ under the provisions of _____

☑ **Other IRB Approved Research Documentation Includes:**

Recruitment Script, Demographic Information Sheet, Recruitment Flyer

☑ **Other Comments:**

Further recruitment materials (letters, advertisements, email texts, etc.) will be reviewed by the IRB Chair on an Expedited basis upon their submission, as directed by the convened IRB meeting on December 4, 2012.

PROTOCOL INFORMATION

Title of Research Activity: **Exercise and NT-020 (NutraStem®): Effects on Stem Cells**

Name of Principal Investigator: **Meharvan Singh, PhD**

Names of each Co-Investigator: **James Simpkins, PhD**

Ralph Anderson, MD, FACOG

Sponsoring Agency / Company (if applicable): **Paula Bickford, PhD, USF Health & Cyndy Sanberg, PhD, Natura Therapeutics, Inc.**

Sponsor's Protocol Number (if applicable): n/a

A. Specific Aims

Determine whether NutraStem® in combination with an exercise stimulus will cause increased human peripheral blood sample levels of CD34+ and CD133+ adult stem cells and whether these stem cells display more protection against oxidative stress.

B. Background and Significance

A stem cell is a specialized kind of cell that has a unique capacity to renew itself and to give rise to specialized cell types. These cells are found in many organs of the adult human including bone marrow, peripheral blood, umbilical cord blood, spleen, tooth pulp, adipose tissue, and brain. Stem cell research is currently a popular subject in the media and science (1). While stem cell therapies have been promised to offer important new treatments for a wide variety of illnesses, these therapies remain controversial and may take years to reach the medical market place due to the lengthy and costly regulatory requirements. Fortunately, a growing body of evidence suggests that there are numerous stem cells continually being produced within the human body throughout the lifespan. These stem cells are located in many different tissues and can become or "differentiate" into virtually any cell type within the body. Internal or endogenous stem cells are crucial to the body's ability to repair itself of degenerating tissues, or to replace cell populations, such as those destroyed by injuries, diseases, disorders, or treatments such as chemotherapy. Healthy stem cells are vital for the body's own natural regeneration and repair mechanisms to function. During natural aging, adult stem cells are known to have a reduced restorative capacity (2, 3) resulting in a reduced ability of the body to heal itself. However, a rapidly growing body of literature now indicates that certain nutrients, vitamins, and flavonoids could have important roles in the proliferation and replacement of stem cells required for healthy self-renewal of mature cells in the blood, brain, and other tissues (4-7). Thus, it appears possible to use certain natural products, either alone or synergistically, for the treatment of conditions where the stem cell replacement appears warranted.

C. Preliminary Studies

Preliminary studies establishing the effect of NutraStem® to increase proliferation of human adults stem cells and to demonstrate a homing effect in

models of injury.

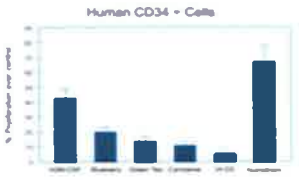

Figure 1. The proprietary combination NT-020 (NutraStem®) promotes proliferation of human CD34+ stem cells in culture and the combination is synergistic as the effect is greater than the 4 ingredients summed. hGM-CSF is included as a positive control. Supplements were added for 72 hours and proliferation assessed by MTT assay.

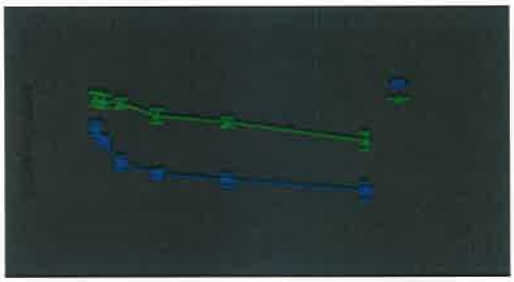

Figure 2. Protection of bone marrow cells in culture in mice fed NT-020 for 2 weeks prior to bone marrow collection. Cells were grown in culture for 3 days and then challenged with varying doses of H_2O_2. In mice receiving either a low or high dose of NT-020 there was significant protection against oxidative damage to the cells.

We have examined the effects of combining different nutritional supplements together with the goal of finding synergistic effects of components to increase actions on the proliferation of progenitor cell populations and to reduce inflammation and oxidative stress. We found that a combination of blueberry, green tea (EGCG), carnosine and Vit D3 (known as NT-020 or NutraStem®) had a synergistic effect to promote proliferation of hematopoietic stem cells in culture (6). In figure 1 is shown the data for CD34+ stem cells and similar data is observed for bone marrow and CD133+ cells. To determine if this proprietary blend would protect cells in vivo NT-020 was then administered to mice for 2 weeks and bone marrow cells removed from the mice and cultured for 3 days (without further NT-020) then exposed to H2O2 to induce oxidative stress. It can be seen that the bone marrow cells from mice treated with either a high or low dose of NT-020 showed protection from the H2O2 insult when cell death was measured by LDH release (Fig. 2). The low dose is equivalent on a mg/kg basis to the human dose available commercially as a dietary supplement, the high dose is actually closer to the biologically equivalent dose as the metabolism of the mouse is approximately 10X higher than human, thus the 135 mg/kg dose is biologically similar to the human dose for expected blood levels of the active ingredients to be used in this study. This also demonstrates that the effect of NT-020 is to modify cell function, perhaps through an epigenetic mechanism as the effect to increase resistance to H2O2 was present 3 days after exposure to NT-020 as the cells were only treated in vivo and NT-020 was not included in the cell culture media.

The next study examined the formulation of NT-020 in an animal model of stroke to examine efficacy in a disease model where we had previously shown that blueberry showed a 50 % neuroprotection from a middle cerebral artery occlusion (MCAO) 60 minute occlusion followed by reperfusion and measured as the size of the infarct when rats were fed blueberry as 2% of the diet beginning 30 days prior to stroke. In this study we examined if the NT-020 would also be neuroprotective and if

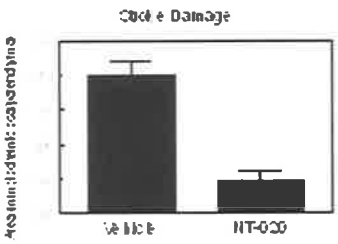

Figure 3. Area of damage following a 60 minute occlusion of the middle cerebral artery (MCAO). Rats treated with NT-0202 show 70% smaller lesion than vehicle treated rats. see Yasuhara et al, 2008

this correlated to an increase in neurogenesis. As can be seen if figure 3 there was a 70% protection of damage following the stroke at 14 days post-stroke in the NT-020 treated animals (7). Animals were treated for 2 weeks prior to stroke (MCAO 60 minute occlusion) and then treatment was not continued after stroke, again demonstrating that the effect outlasts treatment suggesting a change at the cellular level. The next figure (4) demonstrates that this was accompanied by an increase in neurogenesis in both the stem cell niche of the subventricular zone (SVZ) and in the damaged tissue of the striatum. Appropriate controls were performed to analyze double staining of BrDU with doublecortin and the majority of cells labeled with BrDU in the NT-020 treated rats were double labeled with doublecortin, however in the vehicle treated rats the majority of the cells were double labeled with GFAP suggesting a shift of cell fate towards the neuronal lineage following stroke in the NT020 treated rats. Furthermore the data also suggests (but cannot at this time prove) that the homing of the cells from the neurogenic niche (SVZ) to the damaged tissue (striatum) was increased by the treatment. Overall the data show that the combination of blueberry, green tea (EGCG), carnosine and Vitamin D3 is a potent neuroprotective agent that increases neurogenesis. One additional point with this data is that the amount of blueberry used for the individual treatment would be equivalent to about 20 grams of dried blueberry powder per day for human consumption. The synergistic actions of the 4 ingredients combined together showed neuroprotective effects at 1 gram per day human equivalent dosage, thus a reasonable amount of capsules to consume (2 per day) as opposed to 40 capsules per day for the blueberry alone to get an equivalent effect (8).

NT-020 increases neurogenesis in aged rats and improves performance on a Morris water maze.

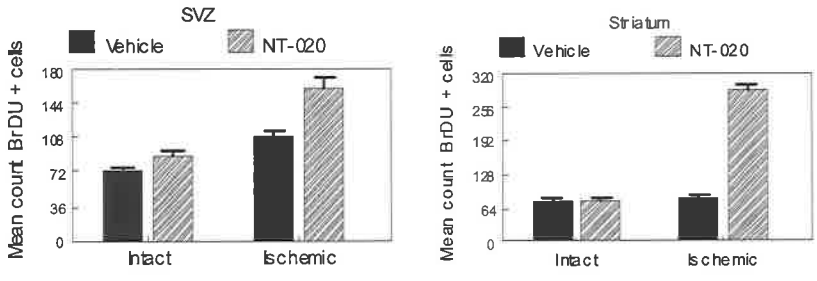

Figure 4. Mean counts of BrdU positive cells in the subventricular zone (SVZ), the neurogenic niche, or the striatum, the damaged area. NT-020 increases BrdU labeling in both regions, with the largest effect observed in the striatum. For specific methods see Yasahura et al Rejuvenation Res. 11:201-214, 2008.

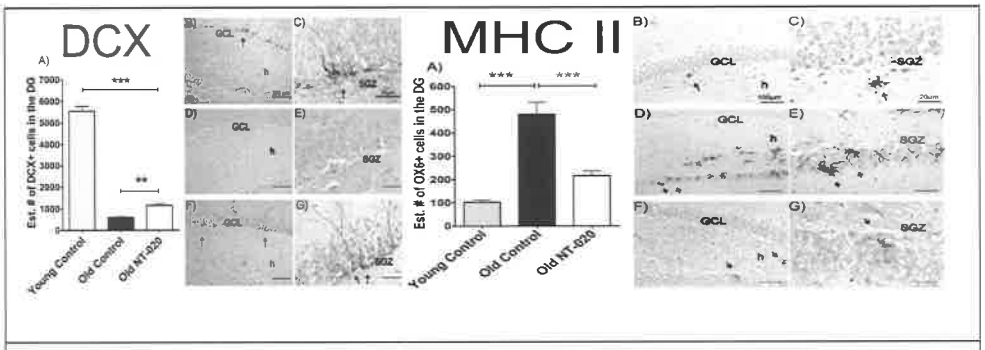

Figure 5: Upper left shows quantification of doublecortin (DCX) positive neuruons in the granule cell layer with inset on the side for young (top), aged (middle) and aged treated (bottom) at low and higher power. Upper right shows quantification for MHCII positive cells – presumably microglia with insert of images as for DCX. Differences were significant at *** p<0.001 or ** p<0.01 using Bonferonni post hocs following one way ANOVA.

As aging has been shown to reduce proliferation of stem cells in many of the stem cell niches of the body we extended our studies to aging models. We examined the effect of NT-020 to improve Morris water maze performance and neurogenesis in the hippocampus and subventricular zone of 20 month old F344 rats (9). Aged (20 month old) male Fischer 344 rats were treated with 135.0 mg/kg per day (n = 13) of NT-020 by oral gavage. Young (3 month old) (n = 10) Aged (20 month old) (n = 13) control male Fischer 344 rats were treated with water by oral gavage. All groups were treated for a period of 4 weeks. We examined learning on a Morris water maze and observed an improvement in memory performance (see Acosta et al, 2010). We next examined proliferation of neural progenitors and microglial phenotype with the diet treatment. Using the cell cycle-regulating protein (Ki67), doublecortin (DCX) and OX6 (MHCII) antibody markers, cell proliferation, neurogenesis and microglial activation were estimated in the dentate gyrus (DG) of young and aged animals. Cell proliferation was also examined in the subventricular zone (SVZ). Decreased number of OX6 MHC II positive cells, increased neurogenesis, and increased number of proliferating cells were found in rats treated with NT-020 in comparison with aged control rats (Figure 5).

D. Investigator Experience

Meharvan Singh, Ph.D. is currently Chair of the department of Pharmacology & Neuroscience, the Director for Research for the Center FOR HER (a women's health center at UNTHSC), and serves as the interim Director of the Institute for Aging and Alzheimer's Disease Research at the University of North Texas Health Science Center. He has had a long-standing interest in the neurobiology of steroid hormones, particularly within the context of brain aging and Alzheimer's disease. He has served as PI of several research grants (including those from the NIH), including a multi-investigator Program Project grant (P01). He continues to successfully administer these projects (e.g. staffing, execution/direction of experiments, managing of budget, etc.), collaborate with other researchers, and ensures the dissemination of his findings through peer-reviewed publications.

E. Experimental Design and Methods

This will be a randomized double blinded placebo controlled trial of a nutraceutical preparation. Up to 60 men and women between the ages of 50 and 70, and generally healthy will participate in this study. All clinical study procedures will occur in the

clinical research lab in CBH 503. This includes informed consent, phlebotomy, blood pressure monitoring, and exercise stimulus. Up to 30 participants will receive the nutritional supplement and up to 30 will receive placebo.

Potential participants will be screened by telephone and have the study explained to them in detail. If they qualify and choose to participate they will come in for the first of three visits. Informed consent will be obtained and demographic information collected. Information requested on the demographic form includes ethnicity, income, education, martital status, smoking and alcohol use, and exercise habits. Next they will have blood pressure checked and if not within parameters they will be dropped from the study and informed that they need to follow up the abnormal result with their health care provider. They will receive $20.00 partial compensation for their time. Next a finger stick blood glucose check will be done to rule out diabetes. If their result falls out of the specified parameter range they will be dropped from the study and informed that they need to follow up the abnormal result with their health care provider. They will receive $20.00 partial compensation for their time. Those that pass these screening tests will have 15 to 25 cc's of peripheral blood (serum) collected by the study coordinator via venipuncture while at rest. They will then stretch for 2 minutes (60 seconds marching in place followed by 10 toe touches) followed by accelerating treadmill walking exercise. The exercise intervention will consist of a 15 minute period of walking on a treadmill. The treadmill will not be inclined during the intervention. The participants target heart rate will be determined by deducting their age from 220. They will start walking on the treadmill at a speed of 1.7 mph and the speed will be titrated as indicated to keep their heart rate at 50 to 90% of target. Heart rate will be monitored via the pulse oximetry feature of the Welch Allyn vital signs monitor. Blood pressure will be measured every 3 minutes during the exercise intervention and until they have returned to baseline (within 10 points) following cessation of the exercise protocol. The test will be stopped for any participant complaint of chest pain, excess fatigue, difficulty breathing, a blood pressure reading of more than 180/94, or a participant request. If symptoms increase or do not completely resolve with 15 minutes of rest the study coordinator will call 911 (through campus police) to activate the EMS response. If symptoms resolve completely within 15 minutes participants will be withdrawn from the study and advised to follow up with their health care provider. Any participant unable to complete the exercise intervention for any reason will be withdrawn from the study.

The exercise intervention is no more intense than what most participants would encounter walking during their normal day-to-day activities. The participants will be closely monitored and the intervention stopped for any subjective or objective evidence of discomfort. Participants involved in this study will have been screened for any medical conditions that would increase their risk of any negative effects from the exercise intervention.

Five to fifteen minutes after the exercise intervention has been completed blood will again be collected in exactly the same manner as the resting sample. Participants will then receive a 2 week supply of either NutraStem or placebo and will be advised to take one tablet every morning and evening before meals for the next 2 weeks. Participants will be advised to bring their empty pill containers to their next session so we can confirm they are taking the supplement as required. Those that complete the 1st session will receive $40.00 compensation. If the participant is unable to complete any portion of the visit activities at any visit they will be withdrawn from the study due to their

inability to complete the study. All visit activities will be recorded in detail on the visit session record.

The second visit will occur 2 weeks later and include a blood pressure check, an at rest blood draw, the exercise intervention, and then a repeat blood draw five to fifteen minutes after completion of the intervention. Participants will then receive a 2 week supply of either NutraStem or placebo and will be advised to take one tablet every morning and evening before meals for the next 2 weeks. I Participants will be advised to bring their empty pill containers to their next session so we can confirm they are taking the supplement as required. If the blood pressure is outside of the specified range they will be dropped from the study and no blood collection or study intervention will take place. They will receive $20.00 compensation for the visit. Those that complete the 2nd session will receive $60.00 compensation.

The third visit will be the same as the second visit. If the session cannot be competed due to failed blood pressure screen participants will receive $20.00. All participants that complete the 3rd session will receive $80.00.

SESSION	ACTIVITIES	VISIT LENGTH	COMPENSATION
VISIT 1	informed consent, demographic information collected, BP screening, diabetes screening, resting blood draw, exercise intervention, post exercise intervention blood draw, receive NutraStem or placebo	40 to 80 minutes	$40.00 *$20.00 if unable to complete session for any reason
Visit 2	resting blood draw, exercise intervention, post intervention blood draw	45 minutes	$60.00 *$20.00 if unable to complete session for any reason
Visit 3	resting blood draw, exercise intervention, post intervention blood draw,	45 minutes	$80.00 *$20.00 if unable to complete session for any reason

Blood Analysis:
A 10ml Lavender top with EDTA tube of collected blood will processed for MNC's and designated for flow cytometry measure the stem cells including CD34+ and CD133+ cells at all blood draw time points. Dr. Stephen Matthew will complete this portion of the analaysis. A 5 ml SSt tube will be collected for a lipid panel on the 1st blood draw of each session and delivered by the coordinator to Quest Lab for analysis. One additional tube of 5 cc's will processed for serum measurements of biomarkers of inflammation and oxidative stress by the sponsor. These measures (IL1 β, IL4, IL6, hsCRP) will be completed by the sponsor. De-identified serum samples will be stored by Dr Singh's' lab in a locked -80 freezer on the 5th floor of CBH until the sponsor specified time points of the study and then shipped to the sponsor following IATA Dangerous Goods Regulations by Dr Singh's lab personnel.

Blood sample collection tubes will be pre-labeled by the pharmacist doing the study drug/placebo randomization. The following randomization system will be used for the samples collected during this study:

Identifier (randomized to active ingredient or placebo)	Subject number	Study Visit	Blood Draw	Description of Blood Draw
JKSB	01	01	01	Pre-exercise
JKSB	02	02	02	Post-exercise
JKSB	03	03	01	Pre-exercise
JKSB	03		02	Post-exercise
JKSB	04		01	Pre-exercise
			02	Post-exercise

Therefore, subject 17 on visit 3 for the post-exercise blood draw would be coded as: JSKB170302

Subject 5 for visit 1 and pre-exercise blood draw would be coded as: JSKB050101

Use of Blood Samples and Related Data For Other Research Purposes In the consent form, subjects will be asked if they agree to the transfer of some of their blood and related research data to other researchers for analysis and testing that may not related to this study (no additional blood will be drawn). This is voluntary, and if subjects do not agree to this transfer, they can still participate in the main study. Blood samples and related data will only be prepared for transfer if the subject meets criteria that is specified by the other researcher in his or her protocol. Prior to transfer, study personnel will de-identify the blood sample and any related data. The specimen container and related data will be labeled with a code that does not identify the subject or contain any personal information about the subject in order to protect his or her confidentiality. Any remaining blood that is not prepared for transfer will be destroyed after the testing for this study is complete. Once transferred, the blood sample and related data will be stored indefinitely by the other researchers. Researchers requesting blood samples and related data from subjects in this study must obtain IRB approval for their study before the PI will initiate the transfer of any samples and related data. Documentation that IRB approval has been obtained will be requested and maintained in the PI's study files. The PI will also provide a letter to the OPHS/IRB that indicates his agreement that the other researcher can use his subject's samples and related data for their research.

Randomization Process (drug):

Dan Hooper, UNT Pharmacist, will create a randomization assignment based on Meinert et al. (10) He will label small envelopes with the STUDY number (Example USF Trial: Subject 1). In this small envelope will be a piece of paper with either the words 'CASE' or 'PLACEBO' or other analogous wording.

These envelopes will be maintained by UNTHSC pharmacist, Dan Hooper. He will keep these confidential.

The pharmacist will keep the product and placebo in separate boxes. The appropriate product/placebo will be placed in a small paper grocery bag and the outside of the bag will be labeled with the STUDY ID NUMBER (Example USF STUDY 12). When the research coordinator is ready for the product/placebo, she will take the bag from the designated area and deliver it to the subject with appropriate instructions.

When additional product/placebo are needed, the same procedure will be followed. All blinding materials will be maintained by Dan Hooper until all data have been collected for the study.

Nutraceutical Information NT-020:
Nutraceutical Information NT-020:

NT-020 (NutraStem® Cardio™) is manufactured locally in the south central Florida region by Nutrition Formulators, Inc., an NSF certified Good Manufacturing Practices (GMP) manufacturer of tablets, capsules, powders and liquids for the nutritional industry. NSF GMP accredidation is one of the most difficult certifications to obtain due to its overall highest levels of standards. NSF is an accredited, third-party certification body that tests and certifies products to verify they meet these public health and safety standards. Products that meet these standards bear the NSF Mark, which is respected by consumers, manufacturers, retailers and regulatory agencies at the local, state, federal and international level. Following is the current GMP certification.

GMP refers to the U.S. Food and Drug Administration's Good Manufacturing Practice Regulations. These are promulgated under the authority of the Federal Food, Drug and Cosmetic Act, and have the force of the law. GMP regulations require a quality approach to manufacturing, enabling companies to minimize or eliminate instances of contamination, lack of uniformity, or poor quality. GMP regulations also address issues including recordkeeping, personnel qualifications, sanitation, cleanliness, equipment verification, process validation, complaint handling, and auditing of performance on a repetitive basis.

Recommended intake of NT-020 is two (2) capsules daily. This comprises Vitamin D3 (2000 IU), BioVin® (40 mg) and the proprietary blend (900mg). This recommended intake was calculated from doses analyzed in scientific research and based on the dose within the submitted patent.

Scientific description of the ingredients of the product per a 2 capsule dose

Proprietary Blend – 900mg:
Vitamin D3 (as cholecalciferol) – 2000 IU
BioVin® Grape Extract – 40 mg
Green Tea Extract (*Camellia sinensis*)
Wild Blueberries* (whole fruit)
Carnosine
VitaBlue® Wild Blueberry Extract*
**Vaccinium angustifolium*

Other Ingredients:
Magnesium stearate, Cellulose (vegetarian capsules)
Contains no yeast, wheat, corn, milk, egg, soy, glutens, artificial colors or flavors, added sugar, starch or preservatives.

Reported Adverse Events and Potential Risks

Although the ingredient combination within NutraStem®'s patented formulation has not undergone human clinical trials at this point, the individual ingredients are well-established as safe with very few adverse effects, side effects, or potential risks. Each ingredient is listed below accompanied by its reported adverse events/side effects. In some instances the dose of that ingredient supersedes the amount within the NutraStem® formulation. This list includes Vitamin D3, BioVin® and the ingredients comprising the proprietary formulation, Green Tea Extract, Wild Blueberry Extract, VitaBlue®, and L-Carnosine.

Vitamin D3 (as cholecalciferol; 2000 IU) Adverse Events:
According to the National Institutes of Health (NIH) factsheet on Vitamin D3, the tolerable levels are 2,000 IU or international units (11). Thus, this study is within the approved levels of Vitamin D3. Vitamin D3 toxicity can cause nonspecific symptoms such as nausea, vomiting, poor appetite, constipation, weakness, and weight loss (12). It can also raise blood levels of calcium, resulting in confusion and heart rhythm abnormalities (13).
The use of supplements of both calcium (1,000 mg/day) and vitamin D (400 IU/day) by postmenopausal women was found to have a 17% increase in the risk of kidney stones over a 7 year period (14). Deposition of calcium and phosphate in the kidneys and other soft tissues can also be caused by excessive vitamin D levels (15). Although Vitamin D3 is absorbed via sunlight, excessive sun exposure does not cause vitamin D toxicity (16).
Long-term intakes above 2,000 IU may increase the risk of adverse health effects (17). Substantially larger doses administered for a short time or periodically (e.g., 50,000 IU/week for 8 weeks) do not cause toxicity. Several nutrition scientists recently challenged these upper levels of Vitamin D3, that were first published in 1997 (18). The more recent clinical trials conducted in healthy adults conclude that the data support an upper level as high as 10,000 IU/day. Although vitamin D supplements ingested above recommended levels given in clinical trials have not shown harm, most trials were not adequately designed to assess harm (19). Currently, there is not enough evidence to determine the potential risks of excess vitamin D in infants, children, and women of reproductive age.

Presently, the Food & Nutrition Board are reviewing data to determine whether updates to the Daily Recommended Intakes (including the Upper Levels) for vitamin D are appropriate.

Potential Risks:

Due to the dose (2,000 IU) of Vitamin D3 within the NutraStem® formulation not exceeding the upper levels as established by NIH, we do not expect to have any adverse effects within the scope of this study. Since the dose of Vitamin D3 within NutraStem® is based on a 70 kg individual or an adult we do not recommend this product to infants, children or pregnant women. Therefore, adverse effects for these groups will not be considerable applicable nor pertinent to this study. Side effects and/or adverse events are not anticipated or expected, but the study coordinator will closely monitor for this possibility.

BioVin® Grape Extract (40 mg) Adverse Events:

In a 2000 clinical study entitled, "Bioabsorption and *In Vivo* Antioxidant Properties of Grape Extract BioVin® : A Human Intervention Study", the following was reported (20). Below is a direct citation from this study.

"Abstract - Recent epidemiological studies have indicated that intake of grapes and other food products such as red wine derived from grapes may lower incidence of cancer and coronary heart diseases. Grapes and grape extracts, in addition to the traditional nutrients such as sugars and vitamins, are also rich sources of polyphenolic antioxidants. A study was undertaken with the overall objective of evaluating the antioxidant potential of the grape extract BioVin® (Cyvex Nutrition, Inc., Irvine, CA) both *in vitro* and in healthy human subjects. A dose-response relationship between the grape extract and its antioxidant potential was observed in the *in vitro* and in liposome system. Fourteen subjects (7 men and 7 women) were orally given BioVin®, 375 mg/day for 4 weeks, followed by a 2-week washout period during which consumption of BioVin® was discontinued. Fasting blood samples were collected at the beginning and end of the treatment period and at the end of the post-treatment washout period. Serum polyphenols, antioxidants, and biomarkers of oxidation including lipid, protein, and low-density lipoprotein (LDL) oxidation were measured. Results showed that BioVin® polyphenols were well tolerated by the subjects and were absorbed readily."

According to additional studies by NIH and the National Center for Complementary and Alternative Medicine (NCCAM), grape seed extract is generally well-tolerated by mouth and has been used safely for up to 8 weeks in clinical trials (21). Side effects that have been reported include a dry, itchy scalp; dizziness; headache; high blood pressure (22); hives; indigestion; and nausea.

Potential Risks:

Since the doses within the above clinical study administering BioVin® far exceed that of the dose within NutraStem® and the finding that even the high doses of BioVin® were well-tolerated for all subjects, we do not expect to have adverse effects reported in this study due to BioVin®. The side effects of increased blood pressure were predominately when grape seed extract was co-administered with Vitamin C in high doses for both (22). Because there is no extra added Vitamin C with NutraStem® and the dose of

BioVin® is much lower than reported side effects, we predict that there will be no adverse effects due to BioVin® . In addition, BioVin® is a full-spectrum grape extract and not just a concentrate of grape seed. Side effects and/or adverse events are not anticipated or expected, but the study coordinator will closely monitor for this possibility.

Green Tea Extract Adverse Events:

According to the NIH, more than five cups per day of green tea is considered "possibly unsafe" due to the side effects of the caffeine (23). These side effects can range from mild to serious and include headache, nervousness, sleep problems, vomiting, diarrhea, irritability, irregular heartbeat, tremor, heartburn, dizziness, ringing in the ears, convulsions, and confusion. In addition green tea seems to reduce the absorption of iron from food (23).

In clinical trials administering caffeinated green tea extracts, cancer patients who had in intake of 6 grams/day, in 3-6 divided doses, experienced mild to moderate gastrointestinal side effects, including nausea, vomiting, abdominal pain, and diarrhea (24,25). Some reported possible central nervous system symptoms, included agitation, restlessness, insomnia, tremors, dizziness, and confusion. These side effects were considered to be due to the caffeine in the green tea extract (25). In a four-week clinical trial that assessed the safety of decaffeinated green tea extracts (800 mg/day of EGCG) in healthy individuals, a few of the participants reported mild nausea, stomach upset, dizziness, or muscle pain (26).

Recently, oral use of green tea extracts (GTEs) has been associated with several instances of hepatotoxicity (27-30). Most affected patients were women, and many were consuming GTEs for the purpose of weight loss. Although hepatotoxicity in most cases resolved within four months of stopping GTE, there have been cases of positive rechallenge and liver failure requiring liver transplantation. A recent case report describes a case of acute liver failure requiring transplant in a woman consuming Green LiteJ capsules distributed by Origin Biomedicinals, Inc., Halifax, Canada (31). Since no other cause could be identified, the treating physicians concluded that the cause of the fulminant liver failure experienced by this subject was most likely related to the consumption of the over-the-counter GTE supplements for weight loss.

Potential Risks:

The amount of green tea extract within NutraStem® has been calculated to be equivalent to 3-4 cups which is less than the amount reported that caused adverse effects according to NIH (23). Most important, however, the percentage of caffeine in NutraStem's green tea extract is considered quite low at a range of 32 – 42 mg per dose. The average green tea on the market has 3.12 mg of caffeine per fluid ounce or 24.96 mg per cup (8 oz.) (32, 33). Thus, the amount of caffeine in NutraStem's green tea extract is less than the amount found in 2 cups of market-grade green tea. Therefore, we do not expect to have adverse effects within this study due to the presence of green tea extract with such low caffeine content. Side effects and/or adverse events are not anticipated or expected, but the study coordinator will closely monitor for this possibility.

Wild Blueberry Extract (Lowbush Powder, VitaBlue®) Adverse Events:

Anecdotal evidence suggests that Blueberry can occasionally induce symptoms of food allergy in sensitized individuals; however, no studies have been reported to date. The lack of reported evidence may be due to the low allergenicity of this berry, the small amounts consumed, or the restricted time frame of consumption. Oftentimes a limited or low exposure to certain allergens might be the reason for the limited complaints reported. However, as the promotion of blueberry consumption continues, this situation may change (34). Nonetheless, based on adverse effects reported to other berries, and particularly to members of the same family, it can be said that blueberries may induce symptoms of food allergy in sensitized individuals (35).

For example, a 25-year-old woman reported adverse reactions due to lingonberry (V. vitis-idaea). While eating lingonberry jam, she developed allergic symptoms including itching wheals around her mouth, however, the symptoms dissipated. During a second episode, when she ingested a very small amount of lingonberry jam several days later, she immediately noticed more-intense symptoms, including severe itching on the mouth, tongue and throat, and wheals over the mouth. Symptoms dissipated within an hour, however, it was noted that upon secondary exposure the symptoms were more severe and a skin reactivity test demonstrated a positive allergic response to lingonberry (36).

Potential Risks:

Since there are no reported adverse effects of blueberries, we expect no adverse effects to occur during this study. Side effects and/or adverse events are not anticipated or expected, but the study coordinator will closely monitor for this possibility.

L-Carnosine Adverse Events:

A double-blind, placebo-controlled study looking at the affects of L-carnosine supplementation in children with autism by Chez et al. (37) showed that the side effects were minimal even at high doses. Thirty-one children (21 M, mean age= 7.45; range = 3.2-12.5 yrs) meeting inclusion criteria were enrolled in an 8 week blinded trial of either 400 mg Carnosine or placebo. Children were assessed at a pediatric neurology clinic with the Childhood Autism Rating Scale (CARS), the Gilliam Autism Rating Scale (GARS), the Expressive and Receptive One-Word Picture Vocabulary tests (E/ROWPVT), and biweekly parental Clinical Global Impression of Change (CGI), at baseline and 8 week endpoint. Results: Children who were on placebo (n=17) did not show statistically significant changes on any of the outcome measures. However, after 8 weeks on L-Carnosine, children (n=14) showed statistically significant improvements on the GARS total score, GARS Behavior, Socialization, and Communication subscales, and the ROWPVT (all p's<.05). EOWPVT and CARS showed trends in improvements, which were supported by parental CGI.

A very small percentage (less than 5% of children with epilepsy or autistic spectrum disorders) showed increased physical hyperactivity or verbal hyperactivity, but whether these side effects were attributable to the carnosine supplement was unclear. No sleep disturbances were reported as a result of carnosine therapy even in dosages up to 3,000 mg a day. No abdominal side effects, skin rashes, or any changes in anticonvulsant blood levels, liver functions or hematological studies were reported. No patients had any urinary changes or bowel habit changes from the carnosine.

Chez et al. (37) reports that manic or hyperactive autistic patients may show signs of over stimulation, including increased irritability, hyperactivity, or insomnia, when given higher doses of L-Carnosine. Symptoms usually respond by decreasing either the dose of L-Carnosine or other medications concurrently. No permanent negative physical changes have been noted in over 1,000 children treated with L-Carnosine since June of 2001. Furthermore, Chez reports no signs of adverse liver, blood, kidney, or central nervous system side effects.

Potential Risks:
Carnosine is considered an extremely non-toxic and safe substance. As with other antioxidants, carnosine acts synergistically when taken with other antioxidants, however, even with this understanding we do not predict any attributable adverse effects to carnosine. Side effects and/or adverse events are not anticipated or expected, but the study coordinator will closely monitor for this possibility.

Rationale for Dose Selection and Administration
We have selected the dose of two capsules per day in this clinical trial. This is based upon our previous experience with the animal models using this compound (Acosta et al.,unpublished observations), as well as previous research that has used concentrated blueberry extract among human (38). Based on these studies, our rationale for dose selection was to maximize bioavailability and the opportunity to observe efficacy, while minimizing toxicity. Participants will be instructed to take the agent in divided doses of one capsule twice a day before meals for 4 weeks.

Availability
NT-020 capsules are an investigational agent supplied by Natura Therapeutics, Inc.

Agent Distribution
Agents will only be released after documentation of IRB approval of the protocol and consent is provided to the sponsor, University of South Florida and the collection of all essential documents is complete.

Agent Accountability
The PI, or a responsible party designated by the investigator, will maintain a careful record of receipt, disposition, and return of all study drugs on the Investigational Agent Accountability Record. All study drug supplies will be kept by the UNT pharmacist, Dan Hooper and study coordinator, Kim Brown, in a locked, limited access area. The study drug will not be used outside the context of the protocol. Under no circumstances will the investigator or other site personnel supply study drug to other investigators, patients, or clinics, or allow supplies to be used other than directed by this protocol. Study agent will not be transferred from one participant to another.
The investigator will maintain records documenting the receipt, use, loss or other disposition of the investigational product, including batch or code numbers, and account for it's disposition on a subject-by-subject basis, including specific dates and quantities.

The source document, documenting the subject's participation in this randomized clinical trial, must be documented in the medical and research records. Destruction will be documented in accordance with institutional SOPs.

Packaging and Labels

NT-020 capsules are packaged with 60 capsules per bottle in 150 cc white HDPE bottles. Bottle labels include agent name, protocol number, dosing and storage instructions, required warnings for restricted, investigational use, and spaces for recording the subject registration and randomization number.

Storage

NT-020 capsules will be stored in a secure location at room temperature [between 59°F and 86°F (15–30°C)].

Side Effects:

All participants will be advised at every visit that if they experience any side effects they feel are related to the supplement they should stop the supplement immediately and consult their health care provider. We will ask that they notify the research coordinator after they have consulted their provider.

Placebo

The placebo is identical in appearance to the NutraStem® supplement and contains the following ingredients in a Vegi Capsule:

MCC200
DICALCIUM PHOSPHATE
BROWN LAKE BLEND (SENSIENT # 09127)
RED DYE DB-088 (COLORCON)
MAGNESIUM STEARATE
BLUE #1 ALUM LAKE (POWDER)

Exercise stimulus

Exercise will consist of 2 minutes of stretching/warm up consisting of marching in place for 60 seconds followed by 10 toe touches. The exercise intervention will consist of a 15 minute period of walking on a treadmill. The treadmill will not be inclined during the intervention. The participants target heart rate will be determined by deducting their age from 220. They will start walking on the treadmill at a speed of 1.7 mphand the speed will be titrated as indicated to keep their heart rate at 50 to 90% of target. Heart rate will be monitored via the pulse oximetry feature of the Welch Allyn vital signs monitor. Blood pressure will be measured every 3 minutes during the exercise intervention and until they have returned to baseline (within 10 points) following cessation of the exercise protocol. The test will be stopped for any participant complaint of chest pain, excess fatigue, difficulty breathing, a blood pressure reading of more than 180/94, or a participant request. If symptoms increase or do not completely resolve with 15 minutes of rest the study coordinator will call 911 (through campus police) to activate the EMS response. If symptoms resolve completely within 15 minutes participants will be withdrawn from the study and advised to follow up with their health care provider. Any participant unable to complete the exercise intervention for any reason will be withdrawn from the study.

The exercise intervention is no more intense than what most participants would encounter walking during their normal day to day activities. The participants will be closely monitored and the intervention stopped for any subjective or objective evidence of discomfort. Participants involved in this study will have been screened for any medical conditions that would increase their risk of any negative effects from the exercise intervention.

Data Analysis/Data Monitoring

All quantifiable results will be expressed as mean ± sem and will be analyzed using repeated measures Analysis of Variance (ANOVA). All post-hoc tests will be conducted using a Scheffè test. Only de-identified data will be sent to the sponsor for completion of data analysis. Subject data will be maintained by the study coordinator in a locked cabinet in CBH 508. Electronic databases will be maintained on a password protected computer. The study coordinator will create and maintain a password protected Excel master list that will contain study participants names, demographic information, study ID, and information recorded during the study sessions. Flow Cytometry results will be submitted to the research coordinator and forwarded from her to the sponsor. Data forwarded to the sponsor will be de-identified.

F. Human Subjects

Up to 60 men and women, generally healthy, age 50 to 70 with no debilitating (defined as a having a chronic disease or condition that has severely limited activities of daily living, normal function, or ability to live an active life or injury). The study coordinator will explain the purpose of the study and describe all visit activities to potential participants. If they are still interested in the study they will be asked questions about their health to determine whether they are able to safely participate in the study. The "screening script" is a written tool based on the inclusion & exclusion criteria that will be used to screen all potential participants.

Inclusion Criteria

- Men or women who are 50 to 70 years of age, inclusive, at the baseline visit.
- Ability to do 15 minutes of treadmill walking exercise.
- Generally healthy

Exclusion Criteria

Subjects who meet any of the following exclusion criteria are not eligible for participation in this clinical research:

- Men and women less than age 50 or more than age 70
- Self reported history of difficult veins/difficulty obtaining blood samples
- Participated in more than 2 sessions per week of strenuous exercise in the last month
    - Are unwilling to follow the procedures of the trial, such as making visits or taking the supplements when asked to;
        - Are unable to tolerate the ingredients in NutraStem® or placebo, or who self report food allergies

- Have unintentionally lost or gained 10 or more pounds of body weight in the last 3 months;
- Have an acute illness (such as a severe cold or flu) or have been hospitalized within the past month.
- Have severe co-morbid disease including cardiac, pulmonary, renal, hepatic, carotid, peripheral vascular disease, stroke, neurological, clotting disorders or active cancer (*defined as any condition that would cause severe limitations or inability to carry out usual activities of daily living)*;
  - Have used any prescription or non-prescription products for antioxidant regimen or stem cell supplement in the 2 weeks prior to beginning this study (Alpha Tocepheral, Vitamin E, Ascorbic Acid, CoQ10)
  - Are diabetic (as defined by diagnosis, use insulin, or a fasting blood glucose value of greater than 120 mg/dl);
  - Have uncontrolled hypertension as evidenced by one of the following;
    - Systolic blood pressure (SBP) > 180 mm Hg or diastolic blood pressure (DBP) > 100 mm Hg, upon two of three repeated measures, if not on medications for hypertension.
    - Systolic blood pressure (SBP) > 150 mm Hg or diastolic blood pressure (DBP) > 90 mm Hg, upon two of three repeated measures, if on medications for hypertension;
  - Have had a recent cardiovascular event (past 36 months), or a family history of sudden death or heart attacks before the age of 55;
  - Have a Body Mass Index (BMI) of less than 20 or greater than 35 m/kg^2;
  - Have participated in a clinical trial in the past 4 weeks;
  - Take methadone, insulin, anticoagulants (blood thinners), MAO's or similar medications;
  - The anticipated need for surgery of any type during the entire study;
  - Subjects who plan to donate blood or blood products during the study or for thirty (30) days following the study;
  - Subjects with evidence of active peptic ulcer disease (vomiting of blood, dark blood in stools or stools that are black or tarry, nausea or vomiting, unexplained weight loss, unexplained appetite changes, abdominal pain) or who have a reliable history of gastrointestinal bleeding within the past five (5) years;
  - Subjects with recurrent or a history of intestinal disorders that may interfere with the absorption of the product (Malabsorption Syndrome, Chrohns, IBS, Ulcerative Cloitis, gastric bypass).
  - Have any disease or condition that in the investigator's opinion compromises the integrity of the clinical trial or the safety of the subject;

The exclusion criteria identified above are based upon general safety concerns identified with the condition and/or product from recommendations made by the study supervisor with input from medical director, confounders identified by the biostatistician, or information identified in product ingredients' research.

Recruitment

Participants who have previously participated in studies with the IAADR (Institute for Aging & Alzheimer's Disease Research) will be sent a letter telling them about the study and inviting them to contact us if they wish to participate. We will also send this letter to people who have called us in the past enquiring about studies and asked us to notify them about future studies. To further recruit qualified participants, we may reach out to the communities in the Metroplex area by use of flyers distributed throughout the community in places suitable for recruitment of participants (e.g., UNTHSC, physicians offices, clinics, health clubs, wellness health conferences, preventive health organizations, public bulletin boards), recruitment letters to men and women in our area identified by City List Co (http://www.citylistco.com/), and the Daily News at UNTHSC. City List Co is 100% HIPAA compliant direct mail participant recruitment service. They work with companies that mail lifestyle surveys. Participants volunteer all information and must "opt-in" for their information to be used. City List will send a tri fold self mailer created from IRB approved flyer content to men and women of the age we specify (per protocol). Possible participants can choose to mail in the perforated reply card or call the Clinical Research Coordinator if they are interested in more information about the research study. The list for the targeted mailing are rented for one time use only and are deleted from City List's hard drive when the mailing is completed. Participants who enroll in the study will be contacted by the study coordinator by phone 24 to 72 hours before each scheduled appointment to remind them of their appointment. This call will remind the participant of the date, time, and location of their appointment ,and remind them not to eat or drink anything but water after midnight the night before their appointment.

G. Risk/Benefit Assessment

Potential Risks

The nutritional supplements being used in this research are commonly available nutritional supplements and are being used in small doses which represents minimal risk to participants. Screening for exclusion criteria will reduce this risk even further. There is no direct benefit to participants.

Blood draws may cause minor pain from the needle insertion, blood clot, or in rare cases, a bruise or infection. These risks will be minimized by the use of standard blood draw precautions.

The exercise intervention will be closely monitored for any participant discomfort by the study coordinator who is a registered nurse. The intervention will be stopped at the request of the participant, & for any reported chest pain, difficulty breathing, blood pressure increased above 180/94, or if the study coordinator believes it is in the best interests of the health of the participant. Blood pressure will be monitored during the rest and recovery phase until it has returned to baseline. If elevated blood pressure, breathing, and/or chest pain do not resolve with 15 minutes of rest or become more severe during that time EMS will be activated by the study coordinator.

Special Precautions

All measures will be taken to protect the confidentiality of participants. All individual data will be coded with a numerical code and will be kept in a locked file cabinet in the

study coordinators office (CBH 508). No other identifying information will appear on the database. All reports and potential publications will report collective information only – participants will not be identified.

Risk/Benefit Assessment
The ingestion of the nutritional supplements involved in this research poses no serious risks to healthy participants. Potential participants will be carefully screened for any medical conditions or medication use that could be impacted by the intake of these supplements. The Modified Bruce protocol was developed for people with poor cardiovascular fitness and our participants will be generally healthy. With the precautions taken to protect participant confidentiality (as mentioned above), the potential benefits of the knowledge expected to be gained from the study outweigh any potential risks.

H. Payment/Compensation
Participants will receive $40.00 for completing the 1st visit, $60.00 for completing the second visit, and $80.00 for completing the final visit to compensate them for their time and discomfort. If participants fail the screening blood pressure or glucose measurements at their first visit they will be dropped and receive compensation of $20.00 for the partial session. If we are unable to successfully complete blood sample collection at any visit they will receive the full compensation for that visit, but will be dropped from the study.

I. **Subject Costs** – There are no costs to the subject to participate in this study.

J. List of KEY PERSONNEL

(1) A. Name and Title: **Meharvan Singh,** PhD
Professor and Chair, Department of Pharmacology and Neuroscience, Interim Director of the Institute for Aging and Alzheimer's Disease Research at UNTHSC.
B. Education, experience, and other pertinent qualifications:
PhD, specialized in research on aging and steroid hormones, including estrogens, progestins and androgens; involved in numerous research projects.
C. Function in carrying out project: Principal Investigator; will be responsible for all phases of the research project. Will coordinate recruitment of study participants obtain informed consents, data analyses, evaluation, and dissemination of results.

(2) A. Name and Title: **James Simpkins,** PhD
Professor, Department of Pharmacology and Neuroscience, Adjunct Professor, Department of Pharmacology & Neuroscience and the Institute for Aging and Alzheimer's Disease Research at UNTHSC, Director of Stroke and Alzheimer's Disease Research West Virginia University Health Science Center

B. Education, experience, and other pertinent qualifications:
PhD, specialized in research on aging and estrogens; involved in numerous research projects.
C. Function in carrying out project: Co-Investigator; will be responsible for assisting in all phases of the research project. Study design and implementation, data analyses, evaluation, and dissemination of results.

(3) A. Name and Title: **Ralph Anderson, MD, FACOG**
Chairman and Professor, Department of Obstetrics and Gynecology, UNTHSC.
B. Education, experience, and other pertinent qualifications:
MD from the University of Western Ontario. Fellow, Royal College of Surgeons of Canada and Fellow, American College of Obstetrics and Gynecology; specialty is Obstetrics and Gynecology Gynecologic Oncology.
C. Function in carrying out project; Medical Advisor

(4) A. Name and Title: **Kimberly Brown,** RN
Clinical Research Coordinator, Department of Pharmacology and Neuroscience UNTHSC.
B. Education, experience, and other pertinent qualifications:
RN, specialized in clinical research; involved in numerous research projects.
C. Function in carrying out project: Clinical Research Coordinator; will be responsible for all phases of the research project. Will coordinate recruitment of study participants, obtain informed consents, perform participant visit functions, coding of data, data entry and maintenance.

K. Literature Cited –

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Attachments (in this order):

I. Consent Form - THE CONSENT FORM IS TO BE A SEPARATE DOCUMENT. It is important that this form follows the IRB prescribed format and includes all the required elements and certain other elements when appropriate.

II. Recruitment Materials (ads, flyers, emails, etc.) to be used in this Study

III. Study Documents (questionnaires, survey instruments, clinical trial protocol, investigator's brochure, etc.)

IV. Evidence of Human Subject Training for ALL Key Personnel listed in the protocol.

V. Conflict of Interest Form, completed and signed by EACH Key personnel listed in the protocol.

INFORMED CONSENT AUTHORIZATION FOR ADULT PARTICIPATION IN A RESEARCH STUDY

TITLE: **Exercise and NT-020 (NutraStem®): Effects on Stem Cells**

SPONSOR: **Paula Bickford, PhD, USF Health & Cyndy Sanberg, PhD, Natura Therapeutics, Inc.**

INSTITUTION: **University of North Texas Health Science Center**

PRINCIPAL INVESTIGATOR: **Meharvan Singh, Ph.D.**
UNTHSC – Fort Worth
Department of Pharmacology and Neuroscience
Institute for Aging and Alzheimer's Disease Research
3500 Camp Bowie Boulevard
Fort Worth, TX 76107
(817) 735-0498

SUBJECT NAME (Please print): _____

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, and relatives if you wish. Take time to decide whether to participate in this research study.

The people who are doing this research are giving you very important information about this research study. When you give your consent for something, it is the same as giving your permission. Please talk to one of the doctors or their staff if you have any questions. Do not sign this consent document unless all of your questions have been answered and you feel comfortable with the information you have read. You will be given a copy of the form to keep.

Before agreeing to take part in this study, it is important to carefully read the following explanation of the intended procedures. This consent form may contain words that you do not understand. Ask the study staff to explain any word or information that is not clear to you.

If you are a student or employee at the University of North Texas Health Science Center, your participation (or non-participation) will in no way affect your academic standing or employment status.

STUDY PURPOSE:

The purpose of this study is to see if an investigational supplement called NutraStem® will increase the amount of stem cells in your blood at rest and after exercise. The ingredients found in NutraStem® can be found in nutrition or vitamin shops/stores.

STUDY PROCEDURES:

You are qualified to participate in this study because you are a generally healthy man or woman age 50 to 70 years old and you are able to walk on a treadmill for 15 minutes.

You may ***not*** participate in this study if you have any of these conditions:

- Age less than 50 or greater than 70 years of age.
- A history of difficult veins/difficulty obtaining blood samples
- Are unwilling to follow the procedures of the trial, such as making visits or taking the supplements when asked to;
- Participated in more than 2 sessions per week of strenuous exercise in the last month
- Are unable to tolerate the ingredients in NutraStem® or placebo, or have food allergies; The ingredients of NutraStem® are:

Proprietary Blend – 900mg:
Vitamin D3 (as cholecalciferol) – 2000 IU
BioVin® Grape Extract – 40 mg
Green Tea Extract (*Camellia sinensis*)
Wild Blueberries* (whole fruit)
Carnosine
VitaBlue® Wild Blueberry Extract*
**Vaccinium angustifolium*
Magnesium stearate, Cellulose (vegetarian capsules)

The ingredients of the placebo are:

MCC200
Dicalcium Phosphate
Brown Lake Blend
Red Dye DB-088
Magnesium Stearate
Blue #1 Alum Lake (POWDER)

Contains ***no*** yeast, wheat, corn, milk, egg, soy, glutens, artificial colors or flavors, added sugar, starch or preservatives.

- Have unintentionally lost or gained 10 or more pounds of body weight in the last 3 months;

- Have an acute illness (such as a severe cold or flu) or have been hospitalized within the past month.
- Have severe co-morbid disease including cardiac, pulmonary, renal, hepatic, carotid, peripheral vascular disease, stroke, neurological, clotting disorders or active cancer (*defined as any condition that would cause severe limitations or inability to carry out usual activities of daily living)*;
- Have used any prescription or non-prescription products for antioxidant regimen or stem cell supplement within the past 4 weeks;
- Are diabetic (as defined by diagnosis, use insulin, or a fasting blood glucose value greater than 120 mg/dl);
- Have uncontrolled hypertension as evidenced by;
    - Systolic blood pressure (SBP) > 180 mm Hg or diastolic blood pressure (DBP) > 100 mm Hg, upon two of three repeated measures, if not on medications for hypertension.
    - Systolic blood pressure (SBP) > 150 mm Hg or diastolic blood pressure (DBP) > 90 mm Hg, upon two of three repeated measures, if on medications for hypertension;
- Have had a recent cardiovascular event (past 36 months), or a family history of sudden death or heart attacks before the age of 55;
- Have a Body Mass Index (BMI) of less than 20 or greater than 35 m/kg^2;
- Have participated in a clinical trial in the past 4 weeks;
- Take methadone, insulin, anticoagulants (blood thinners), MAO's or similar medications;
- Anticipated the need for surgery of any type during the entire study;
- Plan to donate blood or blood products during the study or for thirty (30) days following the study;
- Active peptic ulcer disease (vomiting of blood, dark blood in stools or stools that are black or tarry, nausea or vomiting, unexplained weight loss, unexplained appetite changes, abdominal pain) or who have a reliable history of gastrointestinal bleeding within the past five (5) years;
- Recurrent or a history of intestinal disorders that may interfere with the absorption of the product (Malabsorption Syndrome, Chrohns, IBS, Ulcerative Colitis, gastric bypass).
- Have any disease or condition that in the investigator's opinion compromises the integrity of the clinical trial or the safety of the subject;

During your first visit you will complete the informed consent process. You will be asked to complete a form that collects demographic information about your education, income, marital status, health habits, and relevant health information. Then we will check your blood pressure and blood sugar. If your blood pressure and blood sugar are normal you will be randomly assigned to receive either the supplement or the placebo. If your levels are out of the normal range you will not be able to participate in the study. You will receive $20.00 compensation for your time. If your blood pressure and blood sugar are normal you will be assigned to receive either the nutritional supplement or a placebo. A

placebo is an inactive substance or preparation used as a control in an experiment or test to determine the effectiveness of a medicinal drug. We will not be able to tell you which you are taking until you have completed the research study. You will be provided with a list of possible side effects that you may experience from the nutrition supplement or placebo. We ask that you stop the supplement or placebo if you experience any of these possible side effects and notify the study coordinator. Next we will collect 15 to 25 ml's (3 to 5 teaspoons) of blood. Next, you will be asked to march in place for 60 seconds and attempt to touch your toes 10 times to warm up for walking on the treadmill. After you have warmed up, you will be asked to walk on the treadmill for 15 minutes. The research coordinator will slowly increase the grade and/or speed of the treadmill. If at any time you become uncomfortable you need to tell the research coordinator and the treadmill will be stopped. If you are unable to tolerate walking on the treadmill you will be unable to participate in the study and will receive $20.00 partial compensation for your time. 5 to 20 minutes after you finish walking on the treadmill another 3 to 5 teaspoons of blood will be collected. You will then receive your supplement or placebo for the next 2 weeks. The supplement or placebo should be taken as follows:

1 capsule every morning and 1 capsule every evening before meals

If at any time you feel you are experiencing side effects from the supplement you should stop it immediately and consult your health care provider. Please notify the study coordinator after you have consulted your health care provider.

You will need to fast (nothing but water after midnight the night before your appointment and the morning of your appointment). You may take any regular medications and your supplement the morning of your appointment. Please take them only with water. You will receive $40.00 compensation at the completion of this visit. We ask that you ***NOT*** increase the amount you may exercise while you are participating in this study.

If you are unable to complete any portion of the visit activities at any visit you will be withdrawn from the study as you are unable to complete all activities in the study.

Your 2nd visit will take place 2 weeks after the 1st visit. We ask that you bring your empty supplement/placebo container to this visit. At this visit we will check your blood pressure. If your levels are out of the normal range you will not be able to participate in the study any longer. You will receive $20.00 compensation for your time. If your blood pressure is within normal limits we will collect 15 to 25 ml's (3 to 5 teaspoons) of blood. Then you will be asked to march in place for 60 seconds and attempt to touch your toes 10 times to warm up for walking on the treadmill. After you have warmed up you will be asked to walk on the treadmill for 15 minutes. The research coordinator will slowly increase the grade and/or speed of the treadmill. If at any time you become uncomfortable you need to tell the research coordinator and the treadmill will be stopped. If you are unable to tolerate walking on the treadmill you will be unable to continue participate in the study and will receive $20.00 partial compensation for your time. 5 to 20 minutes after you finish walking on the treadmill another 3 to 5 teaspoons of blood will be collected. You will then receive your supplement or placebo for the next 2 weeks. The supplement or placebo should be taken as follows:

1 capsule every morning and 1 capsule every evening before meals

If at any time you feel you are experiencing side effects from the supplement you should stop it immediately and consult your health care provider. Please notify the study coordinator after you have consulted your health care provider.

You will need to fast (nothing but water after midnight the night before your appointment and the morning of your appointment). You may take any regular medications and your supplement the morning of your appointment. Please take them only with water. You will receive $60.00 compensation at the completion of this visit. We ask that you ***NOT*** increase the amount you may exercise while you are participating in this study.

Your 3rd and final visit will take place 2 weeks after the 2nd visit. We ask that you bring your empty supplement/placebo container to this visit. At this visit we will check your blood pressure. If your levels are out of the normal range you will not be able to participate in the study any longer. You will receive $20.00 compensation for your time. If your blood pressure is within normal limits we will collect 15 to 25 ml's of blood (3 to 5 teaspoons). Then you will be asked to march in place for 60 seconds and attempt to touch your toes 10 times to warm up for walking on the treadmill. After you have warmed up you will be asked to walk on the treadmill for 15 minutes. The research coordinator will slowly increase the grade and/or speed of the treadmill. If at any time you become uncomfortable you need to tell the research coordinator and the treadmill will be stopped. If you are unable to tolerate walking on the treadmill you will be unable to continue participate in the study and will receive $20.00 partial compensation for your time. 5 to 20 minutes after you finish walking on the treadmill another 3 to 5 teaspoons of blood will be drawn.

You will need to fast (nothing but water after midnight the night before your appointment and the morning of your appointment). You may take any regular medications and your supplement the morning of your appointment. Please take them only with water. You will receive $80.00 compensation at the completion of this visit.

The supplements used in this study are:

Proprietary Blend – 900mg:
Vitamin D3 (as cholecalciferol) – 2000 IU
BioVin® Grape Extract – 40 mg
Green Tea Extract (*Camellia sinensis*)
Wild Blueberries* (whole fruit)
Carnosine
VitaBlue® Wild Blueberry Extract*
**Vaccinium angustifolium*

Other Ingredients:
Magnesium stearate, Cellulose (vegetarian capsules) – commonly used in tablet formulations and also found in baby formula.
Contains no yeast, wheat, corn, milk, egg, soy, glutens, artificial colors or flavors, added sugar, starch or preservatives.

Use of Blood Samples and Data For Other Research Purposes: At the end of this consent form, we will ask for your permission to transfer some of the blood that we collect to other researchers for analysis or testing that may not be associated with this study (no additional blood will be drawn). We also will ask for your permission to transfer research data that is associated with your blood sample if it is requested by the researchers for their analysis. Allowing some of your blood sample and data to be transferred to other researchers is voluntary. If you decide not to allow your blood sample and data to be transferred, you can still be in this main study. The research that may be done with your blood sample and related data is not designed to benefit you and will not provide you with any additional information about health conditions you may have. We will only prepare and transfer a portion of your blood sample and associated data if you meet specific criteria that is requested by the other researcher. Your blood sample and any associated data will be de-identified (labeled only with a code that does not identify you or contain any of your personal information) by study personnel before transfer. Once it is transferred, your blood sample will be stored indefinitely by the other researcher(s) in a safe and secure manner. Any remaining blood that is not prepared for transfer will be destroyed after the testing for this study is complete.

RISKS AND DISCOMFORTS OF THIS STUDY:

The risks associated with this study are minimal. This supplement is considered safe for healthy adults. In fact all of the supplements that are in NutraStem® are available without a prescription at health food/vitamin stores.

You may experience a small amount of discomfort from the blood draw where the needle is inserted, blood clot, or in rare cases, a bruise or infection

Special care will be taken to protect your privacy; you will be assigned an identification number and only this number will be used during this study. There may be a very minimal potential for breach of your confidentiality but all necessary measures will be taken to protect your privacy.

All substances have the potential to cause an allergic reaction. If you have ever been told you are allergic to any of these supplements you should not participate in this study. If you experience any symptoms (such as rash, itching, or shortness of breath) that you feel may be related to the supplements you should stop taking them immediately and notify the study coordinator at 817-735-2694.

COSTS AND PAYMENTS FOR BEING IN THE STUDY:

All participants will be compensated $20.00 per visit for partial visits. For each completed visit you will be compensated $40.00 for the 1st visit, $60.00 for the 2nd visit, and $80.00 for the 3rd visit. This is meant to compensate you for time and travel associated with participation in this research study.

CONTACTS:

You may ask questions about this study or your part in this study by calling the Principal Investigator, Dr. Meharvan Singh at the University of North Texas Health Science Center at Fort Worth at 817-735-5429. You may ask about your rights as a participant in research study by calling Dr. Brian Gladue, Chairman, University of North

Texas Health Science Center Institutional Review Board (IRB) at 817-735-0409 for more information. If you think you may be having a problem with any portion of the study, or if you wish to withdraw from the study, contact Dr. Singh by calling 817-735-5429.

BENEFITS:

You will receive no direct benefit from participating in this study. The results from this study will provide us with information about the association between NutraStem® and exercise on the amount of stem cells in human blood.

ALTERNATIVE PROCEDURES:

If you do not want to be in the study, you may choose to not participate.

CONFIDENTIALITY:

We will keep all research records that identify you private to the extent allowed by current local, state and federal law. However, representatives from the Office for Human Research Protection, other governmental regulatory agencies, and the University of North Texas Health Science Center IRB may check you records.

All information which is collected about you during the research will be kept strictly confidential. Any information about you will have your name and address removed so that you cannot be recognized from it. If information about the study is published, it will be written in a way that you cannot be recognized. We will not use your name in any speech or paper about this study.

COMPENSATION FOR INJURY:

We, at the University of North Texas Health Science Center, have not set aside any funds for financial compensation or for costs of medical treatment should you be harmed or injured as a result of your participation in this research.

You should know that by signing this form, you are neither waiving any of your legal rights against nor releasing the principal investigator, the University of North Texas Health Science Center or any of their respective agents from liability for negligence with respect to the conduct of this study.

If you are harmed and you feel that this harm justifies pursuing a legal remedy, then you have the right to do so.

LEAVING THE STUDY:

Taking part in this study is voluntary. It is up to you to decide whether or not to take part in this study. If you decide to take part you are still free to change your mind and quit at any time.

NEW FINDINGS:

You will be informed about any new information that becomes known during this study which might affect your willingness to participate.

CONSENT:

My signature below indicates that I agree to be in this study. I will be given a signed copy of this form. All of my questions about the study have been answered. Being in this study is my choice. After choosing to be in this study, I may quit at any time.

Name of Participant (print)	Signature	Date

Name of Person Conducting Informed Consent Discussion (print)	Signature	Date

PARTICIPATING ON FUTURE RESEARCH STUDIES:

We would like to contact you in the future to see if you would be interested in participating in another research study.
Please indicate by signing your initials below if you are willing to be about any further research studies.

_____ Yes, I agree to be contacted about future research studies.

_____ No, I do not wish to be contacted about future research studies.

TRANSFER OF YOUR BLOOD SAMPLE TO OTHER RESEARCHERS:

Please indicate by signing your initials below if you are willing to allow us to collect one extra teaspoon of your blood for future research and /or the transfer of your blood sample and data to other researchers.

_____ Yes, I agree to allow my blood and related research data (if applicable) to be transferred to other researchers if I meet the criteria that is specified by their research study.

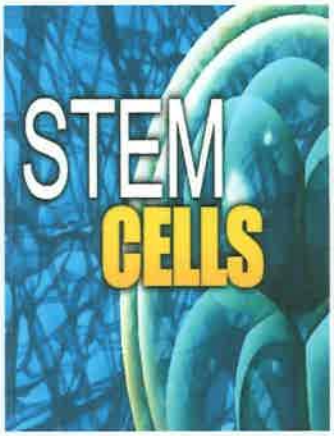
_____ No, I do not want my blood or related research data to be transferred to other researchers and used for other research purposes.

ADDENDUM – SIDE EFFECTS

The individual ingredients in NutraStem® are well-established as safe with very few side effects. However, any substance may potentially cause side effects. The list of possible side effects may rarely be experienced by some people. If you experience any of these possible side effects or nay other unexplained symptoms that you feel may be related to your supplement or placebo please stop the supplement or placebo and notify your health care provider and the study coordinator:

confusion
constipation
convulsions
diarrhea
dizziness
dry itchy scalp
food allergy in sensitized individuals
headache
heart rhythm abnormalities (13).
heartburn
high blood pressure
hives
hyperactivity
indigestion
insomnia
irregular heartbeat
irritability
nausea
nervousness
poor appetite
reduce the absorption of iron from food
ringing in the ears
sleep problems
tremor,
vomiting
weakness
weight loss

Healthy Adults age 50 to 70 are needed for a clinical research study investigating the effect of light exercise and an investigational dietary supplement on your blood stem cells



Researchers at the University of North Texas Health Science Center are investigating whether a dietary supplement combined with light exercise will increase production of stem cells. You may be eligible to participate in this study if:

- **You are ages 50 to 70 years old**
- **You are generally healthy**
- **Able to walk for 15 minutes**

Compensation is provided

Study conducted by The University of North Texas Health Science Center

UNT HEALTH SCIENCE CENTER

For more information please call:

817-735-2694

kim.brown@unthsc.edu

Screening script

How may I help you? If calling about the study;

May I ask your name?:_____

How did you hear about the study? _____

We are running a 4-week study (clinical trial) on normal, healthy people to determine if taking a supplement will help the body produce a very specific type of white blood cells. These are called 'adult stem cells'.

You must be between the ages of 50 and 70 and be in good health with the ability to ride walk on a treadmill at a moderate pace for 15 minutes.

If you choose to enroll in the trial you will be randomized (assigned) to take either the real product or a look-alike placebo (fake-pill).

You take the assigned capsules twice a day for a total of 4 weeks. Every two weeks you will show up to the Clinic and go through an exercise period (for a total of 3 visits).

This consists of having a blood draw before exercise. The exercise consists of a total of 15 minutes walking on a treadmill. Every three minutes the grade will increase slightly. We will be careful not to make the exercise too difficult for you. A few minutes after the completion of the exercise, we will take another blood draw. This will conclude your exercise for the day with us.

At your 1st visit you will read and sign a consent form and give us some demographic information. We will check your blood pressure and glucose and BMI to make sure they are within normal limits. If they are not we will not be able to have you continue in the study. We will compensate you $20.00 for your time if that happens.

If these tests are normal we will collect your blood, have you walk on the treadmill and then collect your blood again. You will receive your supplement of placebo to

take for the next 4 weeks. If you are able to complete this visit you will receive $40.00 to compensate you for time and travel.

If we are unable to collect your blood at any visit we will have to drop you from the study and you will receive partial compensation of $20.00 for your time.

At your 2nd visit we will check your blood pressure & if normal we will collect your blood specimen followed by having you walk on the treadmill again. 5 to 15 minutes after that we will again collect the blood sample. You will receive $60.00 compensation for completing this visit.

At your 3rd & last visit we will check your blood pressure & if normal we will collect your blood specimen followed by having you walk on the treadmill again. 5 to 15 minutes after that we will again collect the blood sample. You will receive $80.00 compensation for completing this visit.

Do you have any questions or concerns? discuss as appropriate

If you are still interested I need to ask you some questions to determine if you qualify to participate in this study. If yes;

Age: _____
(Men/women age 50 to 70)

Difficult blood draw: _____
(exclude if self reported history of difficult veins/difficulty obtaining blood sample)

How much exercise: _____
(exclude if more than 2 sessions per week of strenuous exercise in the last month)

Food allergies_____
(exclude if any)

Diabetic:_____
(exclude if diabetic by diagnosis and/or use insulin or oral diabetic meds)

Hypertension: _____
(exclude if not controlled, under 180/100 not on meds, 150/90 on meds)

Unintentional weight loss/gain: _____
(exclude if lost or gained 10 or more pounds in the last 3 mos)

Current acute cold/flu other: _____
(exclude if yes)

Hospitalization in last month: _____
(exclude if yes)

Chronic illness:

_____
(Exclude if severe co-morbid disease including cardiac, pulmonary, renal, hepatic, carotid, peripheral vascular disease, stroke, neurological, clotting disorders or active cancer (*defined as any condition that would cause severe limitations or inability to carry out usual activities of daily living)*

Antioxidant regimen or stem cell supplement in the 2 weeks prior to beginning this study:

_____
(exclude if took Alpha Tocepheral, Vitamin E, Ascorbic Acid, CoQ10)

Heart issues:

Self: _____ (exclude if cardiovascular event past 36 months)

Family: _____(exclude if family hx sudden death or heart attacks before age 55)

Weight/height: _____ (exclude if BMI less than 20 or greater than 35 m/kg^2)

Other clinical trials: _____ (exclude if participated in a clinical trial in past 4 weeks)

Medications: _____
(exclude if methadone, insulin, anticoagulants, MAO's)

Any surgery planned: _____
(exclude for anticipated need for surgery of any type during study)

Blood donation: _____
(exclude if plan to donate blood or blood products during the study & for 30 days following the study)

Stomach problems: _____

(exclude if evidence of active peptic ulcer disease (vomiting of blood, dark blood in stools or stools that are black or tarry, nausea or vomiting, unexplained weight loss, unexplained appetite changes, abdominal pain)

GI bleeding:_____

(exclude if any hx GI bleed within the past 5 years)

Absorption problems: _____
exclude for:
Malabsorption Syndrome
Chrohns
IBS
Ulcerative Colitis
gastric bypass

If all criteria met and still interested obtain:

Mailing address:_____

Email: _____

Phone #'s: _____

Schedule for session #1 on: _____

Informed consent mailed for review:_____

Remind to fast (nothing but water after midnight) for blood draws

Subject ID: _____ Date: _____/_____/_____

Demographic Form

This questionnaire asks you general demographic questions. We greatly appreciate your help.

1. Your birth-date is: _____,19___, making you: _____ years old

2. Please select your ethnic origin:

□Asian/Pacific Islander □Native American □Black

□White □Hispanic □Other, specify: _____

Your ethnicity:

□Not Hispanic or Latino □Hispanic or Latino

3. Please indicate the category that best describes your current occupation/homemaking status:

□ employed fulltime (36 hours or more) □ employed part-time □ homemaker □ self-employed

□ Disabled □ retired □ student □ not working □Other_____

4. If an alcoholic drink is defined as: one bottle/can of beer equals one glass of wine equals one ounce of hard liquor, **how many drinks do you consume in an average week?**

□ None □ 1 to 2 □ 2 to 3 □ 3 to 4 □ 5 to 6 □ 7 to 8

□ 9 to10 □ more than 10, please specify number: _____

5. Typical choice of alcoholic beverage (beer/wine/liquor):_____

6. Currently smoke cigarettes?: _____ If yes, how many cigarettes per day? _____

Did you ever smoke cigarettes? _____ How many years? _____

7. How many times each week do you exercise? _____ minutes per work out? _____

What kind of exercise? _____

8. In general, would you say your health is:

□ Excellent □ Very Good □ Good □ Fair □ Poor

9. Please circle the **highest** year of school completed:

1 2 3 4 5 6	7 8 9 10 11 12	13 14 15 16	17 18 19 20 21 22	23+
(primary)	(high school)	(college/university)	(graduate school)	

10. Highest degree earned?: _____

11. Marital Status:

☐ Married ☐ Divorced ☐ Widowed ☐ Separated ☐Never Married

12. Total household income per year: (for all adult members of your household)

☐ 0 to $19, 999 per year
☐ $20,000 to $40,000 per year
☐ $40,000 to $60,000 per year
☐ $60,000 to $80,000 per year
☐ $80,000 to $100,000 per year
☐ Over $100,000 per year

Thank you!