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Influence of Benagene Supplementation in Conjunction with High Intensity Cycling Exercise

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The University of Southern Mississippi

Influence of Benagene Supplementation in Conjunction with High Intensity Cycling Exercise

by

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The University of Southern Mississippi
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of the Requirements for the Degree of
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Abstract

High intensity aerobic performance is determined by the body's maximal ability to match lactate production and clearance rates, defined as the lactate threshold (LaTh). Intensities performed above the LaTh result in accumulation of lactate causing fatigue. In a double-blind experiment, using trained cyclists and triathletes, we investigated skeletal muscle adaptations that occurred following 28-days of Benagene supplementation (oxaloacetate). The testing protocol consisted of an initial cycle test (T1) to measure VO_2max, LaTh, respiratory exchange ratio (RER), power output (PO) and heart rate (HR). Testing began after a 15-min warm-up at 75W, beginning at 100W, increasing 30W each 3-min stage until subjects could no longer maintain within 10 rpms of their self-selected pedal rate. Within 3-5 days, subjects performed the second trial (T2) that consisted of a 15-min warm-up at 100 W, followed by a 30-min cycle test to measure power and heart rate at LaTh. Subjects then repeated T1 and T2 within 3-7 days after 28-days of supplementation. Results are inconclusive at this time due to the low subject numbers (3 treatment, 3 control), which resulted from high dropout rates. It was hypothesized that the Benagene supplementation may postpone the accumulation of lactate and therefore increase PO at LaTh.

Key Words: Oxaloacetate, Benagene, Lactate Threshold, Power, VO2, Respiratory Exchange Rate, Heart Rate.

Acknowledgements

I would like to thank my advisor, Dr. Joseph Boyd, for allowing me the opportunity to work on this project alongside him. Dr. Boyd facilitated my introduction into the world of scientific research and exercise science with understanding and ease, inspiring me to continue my education. Because of the time he was willing to invest in me, I have grown as an academic and can confidently pursue a professional career.

Second, I would like to thank Alan Cash for graciously supplying Benagene for this study. Without your donation, this process would have been much more costly and I may not have had the opportunity to work on such an enthralling project.

I would like to thank both my mother and my father, for always believing in me and for always inspiring me to better myself. Without your sacrifices, none of this would be possible.

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List of Abbreviations

AMPK	Adenosine monophosphotase kinase
ATP	Adenosine triphosphate
DEXA	Dual-energy x-ray absortiometry
DNA	Deoxyribonucleic acid
NAD+/NADH	Nicotinamide adenine dinucleotide
VO2 Max	Maximal oxygen uptake
LaTh	Lactate threshold
[La]	Blood lactate concentration

Chapter 1: Introduction

Metabolism

There are several key pathways that contribute to metabolic energy production. Aerobic metabolism, processes completed through the presence of oxygen, tends to produce the high amounts adenosine triphosphate, ATP, allowing for long bouts of exercise. Aerobic metabolism largely consists of glycolysis, the Kreb's cycle, and oxidative phosphorylation.

The aerobic continuation of glycolysis results in the production of pyruvate, a widely useful conjugate base to pyruvic acid which can be altered to form many useful products in regard to the cell metabolism. One of these products, acetyl-CoA, is a precursor for the Kreb's cycle, also known as the Citric Acid cycle. By reducing the molecule via the extraction of hydrogen atoms and the addition of the coenzyme-A group, the conversion of pyruvate to acetyl-CoA allows for effective production of ATP in the Citric Acid cycle.

In conditions where the cell does not have oxygen molecules readily available, such as during high intensity exercise, the systematic fermentation of pyruvate results in the production of lactate. Lactate, an ionized form of lactic acid, accumulates in the cell during these processes due to pyruvate being converted by lactate dehydrogenase. When produced at rates greater than the rates of digestion by muscle tissue, the lactate accumulation can result in acidic pH changes in the blood and tissue, leading to fatigue.

In the aerobic pathway to ATP production, the Kreb's cycle follows glycolysis by converting acetyl-CoA into citrate via the addition of oxaloacetate. Oxaloacetate is a recurring product of the Kreb's cycle and results from the reduction of malate. Oxaloacetate is a point of focus in this study, as the supplement being tested,

Benagene, is chiefly the conjugate acid of oxaloacetate, oxaloacetic acid. Theoretically, an increase in oxaloacetate concentrations during high intensity exercise would reduce lactate accumulation by allowing nicotinamide adenine dinucleotide, NAD+, molecules in the cytosol more opportunity to reduce pyruvate to acetyl-CoA. This would prevent measures of pyruvate from being reduced to lactate via anaerobic fermentation.

During non-vigorous exercise, lactate is produced in muscle tissues and broken down at a similar rate, maintaining homeostasis. When exercise is intensified, there is a gradual increase in muscle lactate concentrations and blood lactate concentrations. The point of onset lactate accumulation is called the lactate threshold. The increase in lactate is largely due to both the hydrolysis of ATP and breaking down of carbohydrates. If the concentration reaches levels that are not sustainable in the muscle during exercise, blood lactate will increase to unbearable intensity, and exhaustion will occur.

Literature Surrounding Oxaloacetate Supplementation

The literature surrounding oxaloacetate is expansive. The body of work surrounding oxaloacetate as a supplement, specifically, is less thorough. The majority of that body of work is composed of research regarding caloric restriction and gene expression. However, the studies linking oxaloacetate to muscle fatigue is nearly non-existent. This is because, only recently, it was inferred that if oxaloacetate relates to more successful aging, then so must it engage a more efficient exercise metabolism. In a study by Nogueira, Hogan and Hogan (2011), oxaloacetate supplementation was found to significantly increase muscle endurance in mouse muscle tissue. Thus, there is some validity to the notion that oxaloacetate

supplementation may increase muscle endurance, though this study is one of very few to explore this topic.

There have been ample studies in regard to the involvement of oxaloacetate with altered gene expression and increased lifespan due to calorie restriction. Calorie restriction, the literal lessening of calories consumed has been directly linked to altering gene expression and extending the human lifespan (Dhahbi et al. 1999). The three pathways identified for altering gene expression via caloric restriction include the activation of adenosine monophosphate kinase (AMPK), the increase in NAD+ inside the mitochondria, and the protection of mitochondrial DNA.

Oxaloacetate has been shown to activate these pathways, as additional oxaloacetate in the cell leads to increasing NAD+ concentrations (Cash 2009). NAD+ has been shown to activate AMPK, which synthesizes DNA, reduces nucleotide cofactors, and oxidizes substrates (Raphealoff-Phail 2004). Thus, by increasing oxaloacetate concentrations, the NAD+/NADH ratio can be increased, AMPK can be activated, calorie restriction can be mimicked, and lifespan can be extended.

It should be noted that oxaloacetate supplementation has been proven to increase lifespan in *C. elegans* through the aforementioned mechanisms, as well (Williams, Cash, Hamadani and Diemer, 2009). Since *C. elegans* is a model species used as a reference for effects on humans, this infers that oxaloacetate supplementation should have similar results on humans.

While there is little research done in regard to oxaloacetate's role in altering muscle endurance, there is ample evidence suggesting that oxaloacetate may be more involved in the body than we know. Based on our knowledge of intracellular metabolism and the balance between aerobic and anaerobic metabolism, we

hypothesize that oxaloacetate supplementation will increase muscle endurance during high intensity cycling.

Chapter 2: Methods

Subject Recruitment

The subjects for the study were volunteers, males' ages 18-44 years and 45-54 years if they were screened and deemed not at risk for hypercholesterolemia and impaired fasting glucose and females' ages 18-54 years**.** The subjects were allowed to partake in the study if they had less than two cardiovascular risk factors outlined by the American College of Sports Medicine. Each subject submitted documents regarding medical history and were asked to complete a physical activity readiness questionnaire (PAR-Q). This research project was approved by the Institutional Review Board for Human Subjects of the University of Southern Mississippi.

Testing Phases

The experimentation occurred in five phases, with the first being a preliminary familiarization session and the following four sessions being analysis during exercise. During the familiarization session, subjects completed an informed consent form, a medical questionnaire, a physical activity readiness questionnaire, measured body height and weight, body composition via DEXA, resting heart rate, lactate and oxygen uptake. Subjects were also introduced to the preliminary stationary cycling protocol, which consisted of a standardizing warm up of fifteen minutes, a practice run of the test, and a recovery stage.

The first experimental tests (T1) occurred within 3-7 days after participants completed the familiarization stage and consisted of tests to determine maximum oxygen uptake (VO_2max) and lactate threshold. Initial steps included measurement of resting heart rate, lactate levels, and oxygen uptake. Following this, the subjects

completed a 10-minute warm up followed by an incremental exercise test for measurement of VO_2max and blood lactate concentration.

For the VO_2max tests, subjects were fitted with a breathing mask connected to a Vyasis Vmax Encore metabolic measurement system. The system software analyzed and reported all major variables, aside from lactate levels. Lactate levels for this study were gathered via 5-microliter blood samples drawn from the earlobe or fingertip. The cycling test began at a resistance of 100W and incrementally increased by 30W every three minutes until the subjects reached volitional fatigue and could no longer continue, as indicated by a decrease of 10 RPMs or more. Immediately, resistance was reduced and a 15-minute recovery ride was completed.

The next test (T2) occurred 3-7 days following the T1 and consisted of a 30-minute test ride at the subjects' established lactate threshold. Blood lactate samples were taken every 4-5 minutes to ensure lactate levels remained constant. Resistance was altered accordingly, to maintain lactate threshold throughout the test. After T2 was completed, the treatment and control groups were established. The treatment group was given a 28-day supply of Benagene and the control received a placebo. The groups were instructed to take two 100mg pills daily with breakfast. Subjects repeated tests T1 and T2, as T3 and T4 within 3-7 days after completion of the 28-day supplementation period.

Chapter 3: Results

Table 1. Characteristics of subjects (n=6)			
Age (yrs)	**Weight (kg)**	**Height (in)**	**BF (%)**
31.88 ± 7.32	80.50 ± 8.36	71.31 ± 2.24	16.99 ± 5.23

Six endurance-trained cyclists and triathletes completed the testing protocol (3 Supplement, 3 placebo) (Table 1). Group 1 consisted of the placebo and group 2 was established as the treatment group. Fifteen subjects began the exercise testing protocol and six completed it successfully. During the testing period, subjects were asked to continue their normal training regimen. Testing included two tests, T1 and T3, for the determination of VO_2max and lactate threshold (LaTh) (Table 2) and two tests, T2 and T4, for the determination of power output and HR at LaTh (LaTh defined as [La] of ~4 mmol/L) (Table 3) during a 30-min cycle test.

Tests T1 and T3 results showed no change or a slight decrease in VO_2max, which was expected due to the short duration of the study and because subjects were well trained. Maximal power output demonstrated an increase in Group 1 and no change in Group 2, which we account for as a greater familiarization to the test and small subject number. Power at LaTh also increased in Group 1 and Group 2, with Group 1 having the greater increase, which was expected due to the greater maximal power output. Results in maximal heart indicated no changes.

Table 2. Results of Test for Maximal Oxygen Consumption, Lactate Threshold and Heart Rate Pre-Post Supplementation				
Test	**VO_2max (ml/kg/min)**	**Pmax (W)**	**P-LaTh (W)**	**HRmax (bpm)**
	Group 1			
T1	54.67 ± 14.4	340 ± 64.8	210 ± 50.9	180.7 ± 11.8
T3	54.0 ± 11.49	390 ± 70.7	270 ± 70.7	180 ± 9.9
	Group 2			
T1	49.8 ± 8.7	370 ± 42.4	260 ± 14.1	180 ± 9.9
T3	53.17 ± 5.8	370 ± 48.9	286.7 ± 9.4	182 ± 6.2

Pmax = maximal power output, P-LaTh = Power at LaTh

Results of tests T2 and T4 to determine power output (P-LaTh) and heart rate at a LaTh (HR-LaTh) of ~ 4 mmol/L are shown in Table 3. Power output at LaTh improved in both groups while HR-LaTh had a slight increase in Group 1 and a slight decrease in Group 2. Again, with such a low number of subjects completing the test, results are inconclusive. No statistics were completed at this time due to the low number of subjects.

Table 3. Results of 30-min Cycle Test at Lactate Threshold Pre-Post Supplementation			
Test	**P-LaTh (W)**	**HR-LaTh (bpm)**	**[La] mmol/L**
	Group 1		
T2	230 ± 57.9	153.3 ± 6.24	3.87 ± 0.12
T4	251.7 ± 77.1	155.6 ± 9.81	3.93 ± 0.12
	Group 2		
T2	258.33 ± 22.5	159.3 ± 14.5	3.8 ± 0.08
T4	265 ± 28.6	155.3 ± 8.1	4.13 ± 0.17

Chapter 4: Discussion

This study was to determine any benefits that Benagene supplementation may have on decreasing the accumulation of lactate and therefore increase the LaTh during high intensity cycling exercise. Any changes that were expected would be attributed to an increased amount of Citric Acid cycle cofactors, as well an increased amount of free NAD+. In the beginning of the Citric Acid cycle, oxaloacetate binds to citrate synthase and creates a binding site for acetyl-CoA, the pyruvate derivative from glycolysis. Citrate is then formed from the mixture and the Citric Acid cycle continues (Williams, Fleck and Deschenes, 2012). By increasing oxaloacetate concentrations, more citrate could theoretically be formed and the cycle could be completed at a greater rate than without supplementation due to the increased ability to use more fatty acids as fuel and less carbohydrates. The decrease in carbohydrate use would be accompanied by a reduction in lactate production. This alone would lead to greater production of energy inside the cellular mitochondria and a greater power output at LaTh.

At the end of anaerobic glycolysis, pyruvate may be reduced to lactic acid due to the need to regenerate NAD+ from NADH. In the Citric Acid cycle, the step that forms oxaloacetate results in the formation of NADH from NAD+ molecules. By increasing oxaloacetate concentrations in the mitochondria, the oxaloacetate-forming step in the Krebs cycle may be bypassed, resulting in a greater amount of free NAD+ and increasing the NAD+/NADH ratio (Cash 2009). The increased amount of available NAD+ could be a reason for reduced lactate production from anaerobic glycolysis, as lactate is formed from pyruvate being reduced by free NADH. However, because we had a greater increase in maximal power and power at LaTh in Group 1 than Group2, and similar changes in both groups, treatment and control, in

power ant LaTh, it is hard to deduce if any differences in performance were observed due to supplementation without increasing the subject pool.

As in any experiment involving human subjects, there is undoubtedly room for error. Due to the time requirements of this study, attrition was expected, but not to the extent observed. With only three subjects in each of the groups, this study was not able to establish any statistical power but does merit the continuation to better establish any possible effects.

Possible future directions could include more in-depth repeats of this study, consisting of more subjects under more controlled circumstances. Because of scheduling conflicts and errors mentioned, many subjects had to withdraw from the program causing our study to lose statistical power. If a study such as this were to be conducted with 18-20 subjects, the power of the results would increase substantially and results could be used to better suggest any possible ergogenic effects.

The implications of this study are of interest to the scientific community for two reasons, athletic gain and increasing lifespan. If the supplementation does indeed increase muscle efficiency, then athletes may benefit from either reducing the use carbohydrates during submaximal exercise, delaying fatigue due to depletion, or increase the use of fatty acids at higher intensities, increasing the LaTh. By simply including this compound in their dietary intake, athletes could train at higher intensities and achieve entirely new feats by being able to endure harsher conditions, for longer.

Chapter 5: Conclusion

Our study has several limitations. First, the experiments were conducted 29-35 days after completion of the second test (T2), which made it difficult to retain subjects due to life events. Second, even though all subjects were trained, not all were equally trained at the intensity that corresponded to LaTh and therefore exercise tolerance at LaTh was more difficult for some. Finally, measuring [La] during an incremental VO_2max test gives you an estimation of [La] at each intensity during each 3-min stage, usually overestimating it. This made it difficult to determine the most precise power output at LaTh for T2 and T4, which required adjusting the intensity during the 30-min cycle test until the correct power output was established. This could be made more precise with at least one more testing session.

In summary, with a greater number of subjects, results may or may not indicate an ergogenic effect, but no conclusion can be made at this time.

Literature Cited

1. Cash, Alan. *Oxaloacetic Acid Supplementation as a Mimic of Calorie Restriction*. Terra Biological LLC. (2009)

2. Dhahbi, J.M., Mote, P.L., Wingo, John, Tillman, J.B., Walford, R.L., Spindler S.R. *Calories and Aging Alter Gene Expression for Gluconeogenic, Glycolytic, and Nitrogen-metabolizing Enzymes*. American Journal of Physiology - Endocrinology and Metabolism (1999) 277, E352-E360.

3. Nogueira, L., Hogan, D., Hogan, M.C. *Acute Oxaloacetate Exposure Enhances Resistance to Fatigue in in vitro Mouse Soleus Muscle*. The FASEB Journal. 2011; 25:1104.56.

4. Raphealoff-Phail, R. *Biochemical Regulation of Mammalian AMP-activated Protein Kinase Activity by NAD and NADH*. The American Society for Biochemistry and Molecular Biology, Inc. 2004.

5. Williams, D.S., Cash, A., Hamadani, L., Diemer, T. *Oxaloacetate Supplementation Increases Lifespan in Caenorhabditis Elegans through an AMPK/FOXO-dependent Pathway.* Aging Cell, 2009 Sep 30.

6. Williams, J.K., Fleck, J.F., Deschenes, M.R. *Exercise Physiology: Integrating Theory n.* Baltimore, MD: Lippincott Williams & Wilkins, 2012.

Appendices

Appendix A

Email Announcement

The Exercise Science program in the Department of Kinesiology is currently looking for trained endurance cyclist or triathletes to participate in an exercise study to look at the possible effects of a nutritional supplement, Benagene, and cycling exercise performance. If you are interested refer to the requirements for participation and contact information below.

Approximately 14-20 participants are being recruited for participation in this study and representation from both genders and all racial and/or ethnic groups is encouraged.

Requirements for participation include:

- Males 18 to 44 and females 18-55 years of age
- Males between 45-54 years of age will be accepted with proof of screening for high cholesterol and diabetes
- Involved in regular aerobic exercise training for the past 6-months at a minimum of 30 minutes per day, 4 days per week
- Must have a Body Mass Index < 30 or body fat percentage < 25%.
- Must be apparently healthy with no indication of risk factors indicated by Health History Questionnaire
- Not taking any medications that might impact exercise metabolism

If you meet the previously listed requirements and are interested in getting more information please contact one of the following individuals:
Wilson Simmons– Wilson.simmons@eagles.usm.edu
Dr. Joseph Boyd – Joseph.Boyd@usm.edu

Appendix B
Recruitment Poster

- WANTED –

RESEARCH SUBJECTS FOR A STUDY INVESTIGATING THE EFFECTS OF BENAGENE SUPPLEMENTATION ON HIGH INTENSITY CYCING EXERCISE

Approximately 14-20 participants are being recruited for participation in this study and representation from both genders and all racial and/or ethnic groups is encouraged.

Requirements for participation include:

- Males 18 to 44 and females 18-55 years of age
- Males between 45-54 years of age will be accepted with proof of screening for high cholesterol and diabetes
- Involved in regular aerobic exercise training for the past 6-months at a minimum of 30 minutes per day, 4 days per week
- Must have a Body Mass Index < 30 or body fat percentage < 25%.
- Must be apparently healthy with no indication of risk factors indicated by Health History Questionnaire.
- Not taking any medications that might impact exercise metabolism

If you meet the following requirement and are interested in getting more information please contact any of the following individuals:

Wilson Simmons– Wilson.simmons@eagles.usm.edu
Dr. Joseph Boyd – Joseph.Boyd@usm.edu
Dr. Michael Webster – Michael.Webster@usm.edu

Appendix C

University of Southern Mississippi
Laboratory of Applied Physiology
Medical History Questionnaire

Directions. The purpose of this questionnaire is to enable the staff of the Laboratory of Applied Physiology to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the **Informed Consent Statement.**

Name: _____ Date of Birth_____

Name of your Physician:_____

MEDICAL HISTORY
Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in blank).

____	Heart murmur, clicks, or other cardiac findings	____	Asthma/breathing difficulty
____	Frequent extra, skipped, or rapid heartbeats	____	Bronchitis/Chest Cold
____	Chest Pain (with or without exertion)	____	Melanoma/Suspected skin Lesions
____	High cholesterol	____	Stroke or Blood Clots
____	Diagnosed high blood pressure	____	Emphysema/lung disease
____	Heart attack or any cardiac surgery	____	Epilepsy/seizures
____	Leg cramps (during exercise)	____	Rheumatic fever
____	Varicose veins	____	Ulcers or digestive disorders
____	Frequent dizziness/fainting	____	Pneumonia
____	Muscle or joint problems	____	Anemia
____	High blood sugar/diabetes	____	Liver (hepatic) or kidney (renal) disease
____	Thyroid Disease	____	Autoimmune disease
____	Low testosterone/hypogonadism	____	Nerve disease
____	Glaucoma	____	Psychological Disorders
____	Chronic swollen ankles	____	Currently Pregnant

To the best of my ability, I will take appropriate measures to prevent my pregnancy while I am a participant in the study. YES NO

Do you have or have you been diagnosed with any other medical condition not listed. YES NO
If yes please list:
_____
Please provide any additional comments/explanations of your current or past medical history that you feel might impact/influence your ability to participate in this study.

_____
_____

Please list any recent surgery that you feel might impact/influence your ability to participate in this study. (i.e., type, dates etc.).

_____
_____

List all prescription/non-prescription medications and nutritional supplements you have taken in the last 3 months.

_____
Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study? YES NO
If yes, please explain (use back side of this page if necessary)

Has your response to any of the above questions changed since the last session in the laboratory?
YES NO

If yes, please explain:

Initials _____ Date _____

Appendix D

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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Appendix E
Informed Consent Statement

ORI | Office of Research Integrity

INSTITUTIONAL REVIEW BOARD

LONG FORM CONSENT

LONG FORM CONSENT PROCEDURES
This completed document must be signed by each potential research participant. • The Project Information section of this form should be completed by the Principal Investigator before submitting this form for IRB approval. • Signed copies of the long form consent should be provided to all participants. Last Edited March 5th, 2014

Today's date:		
PROJECT INFORMATION		
Project Title: **Influence of Benagene Supplementation in Conjunction with High Intensity Cycling Exercise**		
Principal Investigator: Wilson Simmons	Phone: 266-5651	Email: wilson.simmons@eagles.usm.edu
College: COH	Department: Human Performance and Recreation	
RESEARCH DESCRIPTION		

1. Purpose:

High intensity exercise increases the use of carbohydrates and decreases the use of fat for energy, which leads to an increase in Lactate, which causes fatigue. The supplement Benegene may increase the use of fat for energy that is used during high intensity exercise and decrease the use of carbohydrates. This would decrease Lactate and improve performance. The purpose of this study is to give participants a supplement, Benegene, that may reduce the amount of Lactate produced and improve performance while cycling. Bengene as a supplement contains 150 mg of vitamin C and 100 mg of oxaloacetate per capsule.

2. Description of Study:

I am being invited to participate in a research study investigating the use of a commercially available supplement known as Benagene and it's effects on high intensity cycling. All procedures will be conducted at the Laboratory of Applied Physiology at USM. For me to participate in this study I must:

- be male between 18 and 44 or female between 18 and 54 years of age
- be male between 45 and 54 years of age with less than two cardiovascular disease risk factors and also screened and deemed not at risk for both hypercholesterolemia and impaired fasting glucose
- be involved in regular aerobic exercise training for the past 6-months at a minimum of 30 minutes per day, 4 days per week
- must have a Body Mass Index < 30 or body fat percentage < 25%.
- must be apparently healthy with no indication of risk factors indicated by Medical History Questionnaire.
- not taking any medications that might impact normal responses to exercise
- all participants must be healthy, non-pregnant and over 110 lbs.

Approximately 14-20 participants are being recruited for participation in this study and representation from all racial and/or ethnic groups is encouraged.

If at any time during, or after, reading this informed consent, I have any questions or concerns about the document and/or my participation in the study, I am free to ask any of the investigators for additional information or clarification. Once written informed consent is provided, I will perform four separate testing

sessions as follows:

FAMILIARIZATION TESTING SESSION: This will include the measurement or completion of the following:

1. Informed consent
2. a medical history questionnaire
3. a physical activity readiness questionnaire (PAR-Q)
4. Body height and weight
5. Body composition (% body fat determined via Dual Energy X-ray Absorptiometry)
6. Measurement of resting heart rate, lactate and oxygen uptake (VO2)
7. Standardized 10-min cycling warm up
8. Familiarization of cycling exercise test for measurement of oxygen uptake and blood lactate concentration
9. 15 minute active cycling recovery

EXPERIMENTAL TESTING SESSION 1 - T1: Within 3-7 days participants will return to the lab to perform their test for the determination on maximal oxygen uptake and LaTh

1. Measurement of resting heart rate, lactate, and oxygen uptake
2. 10-min cycling warm-up
3. Cycling exercise test for measurement of maximal oxygen uptake and blood lactate concentration
4. 15-min cool-down

EXPERIMENTAL TESTING SESSION - T2: Within 3-7 days participants will return to the lab to perform their first test 30 min ride at LaTh.

1. Measurement of resting heart rate, lactate and oxygen uptake
2. 10-min cycling warm-up
3. 30-min cycling at intensity corresponding to LaTh
4. 15-min cool-down
5. Participants will be given their 28 day supply of either Benagene or placebo (lactose)

EXPERIMENTAL TESTING SESSIONS T3 and T4: Participants will return to the lab for T3 (repeat T1) 29-31 days after completion of T2 and T4 (repeat T2) within 3-5 days after the completion of T3

TABULAR OVERVIEW OF RESEARCH DESIGN

	FS	T1	T2	T3	T4
Medical history questionnaire	X			X	
Physical activity readiness questionnaire (PAR-Q)	X				
Informed Consent	X				
Height, weight, body composition (DEXA)	X				X
Measurement of resting heart rate, Lactate, oxygen uptake	X	X	X	X	X
10 min cycling warm up	X	X	X	X	X
Cycle test for maximal oxygen uptake and blood lactate concentration	X	X		X	
30 min cycling at LaTh			X		X
15min cycling recovery	X	X	X	X	X
PARTICIPANT TIME COMMITMENT (MIN) (TOTAL TIME FOR FS, T1, T2, T3, T4 ~6:30)	90	75	75	75	75

FS - familiarization session

3. **Benefits:**

As a participant in the study, I will receive information with regards to my body composition (% body fat), my maximal oxygen uptake, and my lactate threshold. These are standardized exercise physiology laboratory assessments that can be used in designing a personalize exercise program and are routinely performed in the laboratory. They have a retail value of ~$200. If the potential for medical injury exists, identify treatment procedures or the absence thereof

4. **Risks:**

Exercise Risk - As with any exercise there is health risk. While not an exclusive list, the high intensity nature of the exercise performed in this study subjects me to an increased risk of muscle strains, muscle soreness,

hyperthermia, heart attack, stroke, and in rare instances death. However, the risk of any one or more of these events is minimal among individuals of my age and health status.

No negative long-term effects from Benagene have occurred. Dosages from 100mg to 1,000mg per day have been taken by diabetic patients which showed a reduction in fasting glucose levels at all dosage levels. 100mg was chosen because it has been the lowest level to show a clinical effect in human trials. Toxicity levels have been tested in animals taking up to 5,000 mg/kg with no negative side effects.

Steps taken to minimize my risk from participation:
I am physically trained and of an age that has a relatively low incidence of unknown health abnormalities.

In the event of an emergency, trained personnel will be available to intervene appropriately with CPR skills and in initiating emergency procedures. All of the researchers are CPR/ AEDcertified and two automated external defibrillators (AEDs) are conveniently located in the building. In addition, Dr. Webster is certified with the American College of Sports Medicine as a Clinical Exercise Specialist. A portion of this certification requires successful demonstration of skill in handling emergency situations that may arise in cardiovascular and pulmonary rehabilitation settings.

I am to report to Dr. Joseph Boyd, any unexpected problems or adverse events that I might encounter during the course of the study.

5. **Confidentiality:**

1) All data will be dealt with using a numerical code to identify me as a participant. The coding will only be known by the investigators. My participant information will only be released upon written request to the principal investigator by myself, or in the event of a medical emergency, by the my physician.
2) All data will be on file in the office of one of the investigators and only they will be allowed to examine the data collected on myself.
3) Only group data will be disclosed upon completion and publication of this investigation.
4) Hard copies and electronic copies of data will be kept on file in the office and on the computer of Dr. Boyd for three years, after which time all hard copies will be shredded and all electronic copies will be deleted from hard drives.

6. **Alternative Procedures:**

Not applicable

7. **Participant's Assurance:**

This project has been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations.

Any questions or concerns about rights as a research participant should be directed to the Manager of the IRB at 601-266-5997.Participation in this project is completely voluntary, and participants may withdraw from this study at any time without penalty, prejudice, or loss of benefits.

Any questions about the research should be directed to the Principal Investigator using the contact information provided in Project Information Section above.

CONSENT TO PARTICIPATE IN RESEARCH

Participant's Name:

Consent is hereby given to participate in this research project. All procedures and/or investigations to be followed and their purpose, including any experimental procedures, were explained. Information was given about all benefits, risks, inconveniences, or discomforts that might be expected.

The opportunity to ask questions regarding the research and procedures was given. Participation in the project is completely voluntary, and participants may withdraw at any time without penalty, prejudice, or loss of benefits. All personal information is strictly confidential, and no names will be disclosed. Any new information that develops during the project will be provided if that information may affect the willingness to continue participation in the project.

Questions concerning the research, at any time during or after the project, should be directed to the Principal Investigator with the contact information provided above. This project and this consent form have been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations. Any questions or concerns about rights as a research participant should be directed to the Chair of the Institutional Review Board, The University of Southern Mississippi, 118 College Drive #5147, Hattiesburg, MS 39406-0001, (601) 266-5997.

The University of Southern Mississippi has no mechanism to provide compensation for participants who may incur e injuries as a result of participation in research projects. However, efforts will be made to make available the facilities and professional skills at the University. Participants may incur charges as a result of treatment related to research injuries. Information regarding treatment or the absence of treatment has been given above.

_____	_____
Research Participant	**Person Explaining the Study**
_____	_____
Date	**Date**

Appendix F
Institutional Review Board

THE UNIVERSITY OF SOUTHERN MISSISSIPPI

INSTITUTIONAL REVIEW BOARD
118 College Drive #5147 | Hattiesburg, MS 39406-0001
Phone: 601.266.5997 | Fax: 601.266.4377 | www.usm.edu/research/institutional.review.board

NOTICE OF COMMITTEE ACTION

The project has been reviewed by The University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the "Adverse Effect Report Form".
- If approved, the maximum period of approval is limited to twelve months.
  Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: 14111901
PROJECT TITLE: Influence of Benagene (OAA) Supplementation in Conjunction with High Intensity Cycling Exercise
PROJECT TYPE: New Project
RESEARCHER(S): Wilson Simmons, Joseph Boyd, Ph.D. and Michael Webster, Ph.D.
COLLEGE/DIVISION: College of Health
DEPARTMENT: Human Performance and Recreation
FUNDING AGENCY/SPONSOR: N/A
IRB COMMITTEE ACTION: Full Committee Review Approval
PERIOD OF APPROVAL: 03/16/2015 to 03/15/2016
Lawrence A. Hosman, Ph.D.
Institutional Review Board