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Clinical safety assessment of oral higenamine supplementation in healthy, young men

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Abstract

Objective: Higenamine, an herbal agent also known as norcoclaurine, is thought to stimulate β-androgenic receptors and possess lipolytic activity. It is currently making its way into the dietary supplement market. To our knowledge, no studies have been conducted to determine the safety profile of oral higenamine when used alone and in conjunction with other commonly used lipolytic agents.

Methods: Forty-eight men were assigned to ingest either a placebo, higenamine, caffeine, or higenamine + caffeine + yohimbe bark extract daily for a period of 8 weeks. Before and after 4 and 8 weeks of supplementation, the following variables were measured: resting respiratory rate, heart rate, blood pressure, urinalysis, complete blood count, metabolic panel, liver enzyme activity, and lipid panel.

Results: No interaction effects were noted for any variable ($p > 0.05$), with no changes of statistical significance occurring across time for any of the four conditions ($p > 0.05$).

Conclusion: This is the first study to determine the safety profile of oral higenamine intake in human subjects. Our data indicate that 8 weeks of daily higenamine supplementation, either alone or in conjunction with caffeine and yohimbe bark extract, does not result in a statistically significant change in any of the measured outcome variables. Additional studies, inclusive of a larger sample size, are needed to extend these initial findings.

Keywords

Higenamine, norcoclaurine, dietary supplements, caffeine, safety

Introduction

Higenamine, also referred to as norcoclaurine, is a plant-based compound used in Chinese herbal medicine. Higenamine is found naturally in *Aconitum japonicum* (root)[1,2] as well as in other plants.[3] This1-benzyl-1,2,3,4-tetrahydroisoquinoline alkaloid has been used to improve cardiac left ventricular function[4] and to stimulate β-adrenergic receptors (β-AR).[5] It has also been suggested to yield vasorelaxation properties[6] and to impart antiplatelet aggregation.[7,8]

Due to the above-stated effects, some believe that intravenous higenamine may be a candidate agent for use in pharmacological stress testing (in conjunction with myocardial perfusion imaging). In addition, oral use of higenamine is now beginning to receive attention from the dietary supplement community, as a potential agent to be included within weight loss and sport performance supplements.

It has been reported that β-AR agonists enhance lipolysis and thermogenesis,[9] and we have recently noted an acute increase in both lipolysis (documented via an increased appearance of blood free fatty acids) and energy expenditure when a higenamine-based dietary supplement (higenamine + caffeine + yohimbe (HCY) bark extract) was ingested by men and women.[10] These effects may be partly ascribed to the caffeine[11] and yohimbe[12,13] content of the supplement, as both agents have been reported to have

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lipolytic and thermogenic activity. In this same study, it was noted that both resting heart rate (approximately 3 beats min^{-1}) and systolic blood pressure (approximately 12 mmHg) were elevated following acute intake of the supplement, which may have been due in part to the caffeine and yohimbe content.

To our knowledge, no published human studies have been conducted to determine the safety profile of oral higenamine, alone and in conjunction with other lipolytic agents, following multiple weeks (eight) of intake. With the exception of our recent work involving oral intake of higenamine, we are aware of two published human studies that have determined the hemodynamic effects of higenamine following a single use.[14,15] In the first study,[14] 10 subjects received continuous, *intravenous infusion* of higenamine at gradually escalating doses from 0.5 to 4.0 $\mu g\,kg^{-1}\,min^{-1}$, with each dose provided for 3 min. Heart rate, blood pressure, and other commonly obtained clinical data were collected from subjects, with no events being noted as more than transient (e.g. moderate dizziness and nausea). An increase in systolic blood pressure was noted (mean baseline value of 109 mmHg; maximum 15 min post-dose value of 130 mmHg), while a decrease in diastolic blood pressure was also apparent (mean baseline value of 67 mmHg; minimum 11 min post-dose value of 51 mmHg). In the second study,[15] 10 subjects received *intravenous administration* of higenamine at a dosage of 22.5 $\mu g\ kg^{-1}$, with no adverse events reported. A study using dogs found no significant increase in systolic blood pressure and a decrease in diastolic blood pressure following intravenous administration of higenamine.[16]

Considering the relative paucity of data pertaining to intake of higenamine by human subjects, additional study is warranted to assess the clinical safety profile of *oral* higenamine. This should be considered in light of the fact that (1) the work by Feng and colleagues involved single uses of intravenous higenamine;[14,15] (2) our prior work involving oral ingestion of higenamine only involved a single dose; (3) no measures of clinical safety aside from heart rate and blood pressure were included in our prior work; and (4) our prior work did not include higenamine in isolation, which leads to the possibility that the heart rate and blood pressure elevation was a function of the caffeine and yohimbe bark extract. Because higenamine is now making its way into the dietary supplement marketplace and no readily available human oral intake toxicology studies have been performed to date, it is both of interest and timely to generate safety data for this agent.

Methods

Purpose and design

This study sought to determine the safety profile of higenamine when used alone and in conjunction with caffeine and yohimbe bark extract—two commonly used lipolytic dietary agents (and two agents that were included in our prior work[10]). A caffeine only condition was used as a positive control, in addition to a placebo condition. Outcome measures of clinical safety included resting respiratory rate, heart rate, blood pressure, urinalysis with microscopic examination, complete blood count, metabolic panel, liver enzyme activity, and lipid panel.

Subjects

A total of 51 men initially signed consent to participate in this study. Two of these men (one assigned to placebo and the other to caffeine) decided to cease participation due to personal reasons. One man (assigned to caffeine) decided to end his participation after 4 weeks because he mentioned that he enjoyed the "feeling" that the supplement provided to him and was concerned that he might become addicted to the supplement if he continued using. Data for these men are not included in the analysis. Three additional subjects were enrolled to fill the slots of these subjects, in order to maintain an equal sample size within each condition. Therefore, a total of 48 men completed the study and were included in the final analysis. Subject baseline characteristics are presented in Table 1.

Men were recruited to participate via informal word of mouth conversations, e-mail communications, and recruitment flyers posted on campus. All subjects' recruitment was performed by the investigators (JMS and TAG). Women were not enrolled in this study in an attempt to maintain a more homogenous sample. Subjects were not current smokers and were considered to be in good overall health, without a history of cardiovascular, neurological, or metabolic disorders (e.g. hypertension, seizures, and diabetes). All subjects were regular consumers of stimulants (e.g. caffeine) within beverages or nutritional supplements who did not report a history of adverse reactions to stimulant use.

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Table 1. Characteristics of healthy men assigned to placebo, higenamine, caffeine, or HCY for 8 weeks.[a]

Variable	Placebo ($n = 12$)	Higenamine ($n = 12$)	Caffeine ($n = 12$)	HCY ($n = 12$)
Age (years)	23.4 ± 2.7	23.7 ± 3.7	26.3 ± 5.3	24.3 ± 4.3
Height (cm)	179.1 ± 8.6	179.1 ± 3.7	177.2 ± 5.9	176.6 ± 6.3
Body weight (kg)	79.2 ± 9.6	89.0 ± 17.4	87.1 ± 13.5	82.0 ± 14.3
Body mass index ($kg\,m^{-2}$)	24.7 ± 3.2	27.8 ± 5.3	27.6 ± 2.9	26.3 ± 4.5
Waist circumference (cm)	81.8 ± 6.9	86.5 ± 12.4	89.4 ± 10.0	86.3 ± 13.4
Hip circumference (cm)	99.4 ± 6.3	105.9 ± 8.0	104.7 ± 8.6	101.5 ± 7.0
Waist:hip	0.82 ± 0.05	0.81 ± 0.04	0.85 ± 0.05	0.85 ± 0.09
Weekly aerobic training (h)	2.8 ± 3.5	3.2 ± 2.5	2.8 ± 1.6	2.3 ± 1.6
Aerobic training history (years)	3.1 ± 3.1	5.4 ± 5.4	6.8 ± 5.7	5.8 ± 5.0
Weekly anaerobic training (h)	3.6 ± 3.2	3.5 ± 2.7	2.1 ± 2.3	2.5 ± 1.8
Anaerobic training history (years)	3.4 ± 3.0	4.6 ± 3.4	4.1 ± 5.8	4.5 ± 4.4

HCY: higenamine + caffeine + yohimbe.
[a]Values are mean ± SD. No differences of statistical significance noted ($p > 0.05$).

Health history, medication and dietary supplement usage, and physical activity questionnaires were completed by all subjects and reviewed by an investigator to determine eligibility. Subjects were informed of all procedures, potential risks, and benefits associated with the study through both verbal and written form. The study procedures were approved by the University Institutional Review Board (IRB) for Human Subjects Research (protocol #2601). All subjects who completed the study were compensated US$200 for their time and effort.

Testing and supplementation

During the initial lab visit, subjects completed all paperwork, including the informed consent. Subjects then returned to the lab on three occasions (before supplementation, after 4 weeks of supplementation, and after 8 weeks of supplementation) to complete the assessments indicated below. On each occasion subjects reported to the lab following a minimum 10-h overnight fast. Upon arrival:

1. Subjects voided. They were given a standard urine collection cup and provided with instructions for filling. Urine was stored in a refrigerator and analyzed within 24 h for a complete urinalysis with microscopic examination.
2. Subjects rested quietly for 10 min while seated in a chair.
3. Respiratory rate (in 60 s) was counted by observation.
4. Heart rate (in 60 s) was counted by palpation of the radial artery.
5. Blood pressure was measured by two trained technicians using a stethoscope and cuff.
6. A blood sample was taken and processed as described below.

Following the above assessments on day 1, subjects were randomly assigned to one of four conditions, in double-blind manner: placebo (cellulose), caffeine (125 mg per capsule), higenamine (50 mg per capsule), or higenamine (50 mg per capsule) + caffeine (125 mg per capsule) + yohimbe bark extract (3.5 mg per capsule). Subjects were allocated into groups until each group included a maximum of 12 subjects, in order to maintain an equal sample size within each condition. All conditions were produced under standard good manufacturing practices by a dietary supplement contract manufacturer. Quality assurance procedures confirmed the purity and potency of each condition.

For all four conditions, subjects were instructed to ingest between one and three capsules every day of the week, for a total of 8 weeks, as indicated below. Subjects were instructed to ingest one capsule daily (within 1 h of waking) for the first 3 days. This was to allow subjects to become familiar with the condition. Beginning on day 4 and continuing until the end of the study period, subjects were instructed to ingest two capsules per day in divided dosages (6–8 h apart: the first capsule within 1 h of waking). If well tolerated and the subject chose to do so, they were instructed that they may ingest a third capsule daily (taken with the morning capsule). Therefore, the maximum capsule intake was three daily. In the event that subjects felt best ingesting only one capsule daily, they were allowed to do so. This "freedom" to

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determine capsule dosing was done in an attempt to mimic real-world use of such supplements. The final capsules were to be consumed on the day prior to the final test day; no capsules were consumed before assessments were performed on the mornings of test days. Although the same instructions for use were provided to subjects in all four conditions, no instruction was provided regarding whether the capsules needed to be ingested before or after meals. Therefore, subjects may have ingested the capsules on an empty stomach or in a fed state. This may be considered a limitation of this work, as the possibility exists that the absorption of the ingredients may have varied depending on the stomach contents during the time surrounding capsule ingestion. Future studies may seek to provide specific instructions to subjects with regard to the time of food ingestion relative to capsule intake.

Subjects were instructed not to ingest capsules within 8 h of bedtime, as it was believed that this may have induced sleeplessness. In addition, subjects were advised not to consume caffeinated beverages such as ''energy drinks'', coffee, tea, or soda during the study period and not to use other dietary supplements containing caffeine or other stimulants. Analysis of subjects' self-reported capsule intake indicated that all subjects ingested either two or three capsules daily, except for one subject assigned to placebo, two subjects assigned to higenamine, and one subject assigned to HCY. Capsule intake data were not available for one subject assigned to higenamine. Subjects ingested the following number of capsules daily throughout the study period: placebo (2.25 ± 0.18), caffeine (2.25 ± 0.13), higenamine (2.0 ± 0.19), or HCY (2.25 ± 0.18). This intake did not differ between conditions ($p = 0.68$).

Blood and urine collection and analysis

Venous blood samples (approximately 15 mL) were taken from subjects via needle and Vacutainer® (Becton Dickinson, Franklin Lakes, New Jersey). Blood samples were collected before starting supplementation (pre) and after 4 and 8 weeks of supplementation. Following collection, samples were processed, and fresh samples were analyzed for complete blood count (Coulter LH750, Beckman Coulter, Inc.), comprehensive metabolic panel (Roche/Hitachi Modular), γ-glutamyl transferase (GGT; kinetic methods), and lipid panel (Roche/Hitachi Modular). Morning urine samples were collected in plastic containers and stored in a refrigerator until analyzed for complete urinalysis with microscopic examination (reagent strip and light microscopy).

Dietary intake and physical activity

All subjects were instructed to maintain their normal diet throughout the study period. Subjects were asked to maintain their usual physical activity patterns during the entire course of the study but to avoid strenuous physical activity for the 48 h prior to each test day.

Statistical analysis

Data were analyzed using a four (condition) by three (time) analysis of variance. Tukey's post hoc testing was used as needed. The data are presented as mean $\pm$ SEM, with the exception of subject characteristics (mean $\pm$ SD). All analyses were performed using JMP statistical software (Version 4.0.3 (SAS Institute Inc. Cary, NC)). Statistical significance was set at $p \leq 0.05$.

Results

A total of 48 subjects successfully completed the 8-week study (12 subjects within each condition). However, selected data from the complete blood count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) were not available for the 4-week assessment time for one subject assigned to the caffeine condition.

Two adverse events were recorded and submitted to our IRB for consideration. They are as follows: (1) One subject assigned to the caffeine condition experienced a rash on both arms on the 5th day of treatment. The rash did not affect the subject other than being irritating. The subject reported that he was outside in the sun for an extended period of time and wearing a sleeveless shirt for 2 days prior to the onset of the rash. The rash subsided within a few days of onset and the subject completed the study without incident. (2) One subject assigned to the HCY reported a lack of appetite, a rapid heart rate, difficulty falling asleep at night, and bad dreams within the first few days of treatment. The subject indicated that he accidentally consumed double the dosage that was indicated. After speaking with investigators, he indicated that he wanted to continue with the study and did so without incident. No other adverse events were noted for any subject.

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Table 2. Cardiorespiratory data of healthy men assigned to placebo, higenamine, caffeine, or HCY for 8 weeks.

Variable	Placebo ($n = 12$)	Higenamine ($n = 12$)	Caffeine ($n = 12$)	HCY ($n = 12$)
Respiration (breaths min^{-1})				
Pre	11.9 ± 1.1	12.4 ± 0.8	12.1 ± 1.2	12.2 ± 1.0
4 Weeks	10.8 ± 1.1	11.6 ± 0.8	13.3 ± 1.3	12.4 ± 1.5
8 Weeks	12.0 ± 1.1	12.8 ± 1.1	12.5 ± 0.9	12.6 ± 0.9
Heart rate (beats min^{-1})				
Pre	61.8 ± 2.2	66.7 ± 2.9	67.6 ± 3.3	68.2 ± 3.4
4 Weeks	65.8 ± 3.1	70.1 ± 3.6	67.6 ± 3.5	65.0 ± 2.8
8 Weeks	63.9 ± 2.6	67.0 ± 3.1	68.7 ± 3.5	69.6 ± 2.7
SBP (mm Hg)				
Pre	109.8 ± 2.6	110.0 ± 3.1	108.6 ± 2.7	104.2 ± 1.3
4 Weeks	105.3 ± 2.0	109.2 ± 3.0	108.5 ± 2.5	104.9 ± 3.3
8 Weeks	104.3 ± 2.8	105.3 ± 1.8	108.5 ± 2.6	107.5 ± 3.0
DBP (mm Hg)				
Pre	62.2 ± 1.8	65.8 ± 2.4	67.4 ± 3.2	63.8 ± 2.6
4 Weeks	65.7 ± 2.1	66.4 ± 3.0	67.1 ± 3.0	66.4 ± 2.9
8 Weeks	63.3 ± 3.0	62.2 ± 2.4	66.0 ± 3.6	63.1 ± 3.0
Rate pressure product				
Pre	6761.4 ± 246.4	7366.5 ± 428.7	7363.8 ± 436.3	7114.9 ± 388.8
4 Weeks	6940.8 ± 386.5	7689.8 ± 447.9	7350.2 ± 488.0	6867.8 ± 447.6
8 Weeks	6675.3 ± 343.8	7079.8 ± 505.8	7513.3 ± 381.5	7499.2 ± 401.2

HCY: higenamine + caffeine + yohimbe; SBP: systolic blood pressure; DBP: diastolic blood pressure.
[a]Values are mean ± SEM. No differences of statistical significance noted ($p > 0.05$).

When considering all variables, no interaction effects were noted ($p > 0.05$). In addition, very little change was noted across time for any variable and no time effects were observed ($p > 0.05$). Several condition effects were noted ($p < 0.05$), mostly due to baseline (pre supplementation) differences in subjects assigned to the four conditions. The noted condition effects are indicated within each result table. Cardiorespiratory data are provided in Table 2. Complete blood count, metabolic panel, and lipid panel data are provided in Tables 3, 4, and 5, respectively. Urinalysis data (not shown) were nearly identical between conditions and across time ($p > 0.05$).

Discussion

Findings from this study indicate that 8 weeks of supplementation with higenamine, either alone or in conjunction with caffeine and yohimbe bark extract, does not result in a statistically significant change in any of the measured outcome variables. These data are specific to a sample of healthy, young men consuming moderate daily dosages of the tested ingredients—with respect to the chosen outcomes measures only. Specifically, based on capsule intake data, subjects ingested approximately two capsules daily in all conditions. Based on our data, coupled with those of Feng and colleagues,[14,15] it appears that supplementation with higenamine is well tolerated by human subjects. Of course, additional study is welcome, perhaps involving a longer time course of treatment with higenamine, as well as the use of different dosages of higenamine, in order to extend our findings. Related to dosing, due to the fact that most subjects ingested either two or three capsules per day of the assigned condition, coupled with the fact that our sample size of 12 subjects per group was relatively small, we were unable to perform an analysis comparing different dosages of the assigned conditions (1, 2, or 3 capsules per day). It should be noted that this was not the purpose of this study but may be the focus of future work involving higenamine.

All collected measures in this study were unremarkable, with values remaining very stable across time for subjects in all conditions (Tables 2 to 5). Several condition effects were noted, most of which appear due to differences at baseline between the four conditions. One difference that was not of

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Table 3. Complete blood count data of healthy men assigned to placebo, higenamine, caffeine, or HCY for 8 weeks.[a]

Variable	Placebo ($n = 12$)	Higenamine ($n = 12$)	Caffeine ($n = 12$)	HCY ($n = 12$)
WBC ($10^3\,\mu L^{-1}$)				
Pre	5.1 ± 0.2	5.3 ± 0.4	4.9 ± 0.4	5.9 ± 0.5
4 Weeks	5.3 ± 0.4	6.0 ± 0.5	5.2 ± 0.4	5.5 ± 0.5
8 Weeks	4.9 ± 0.4	5.3 ± 0.4	5.2 ± 0.4	5.8 ± 0.4
RBC ($10^6\,\mu L^{-1}$)				
Pre	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.1 ± 0.1
4 Weeks	4.9 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
8 Weeks	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
Hemoglobin ($g\,dL^{-1}$)				
Pre	14.7 ± 0.2	14.8 ± 0.2	15.1 ± 0.3	15.0 ± 0.3
4 Weeks	14.9 ± 0.1	14.7 ± 0.2	14.9 ± 0.3	14.8 ± 0.3
8 Weeks	14.9 ± 0.2	14.9 ± 0.3	15.0 ± 0.3	14.8 ± 0.3
Hematocrit (%)				
Pre	44.0 ± 0.6	44.3 ± 0.6	44.6 ± 0.9	44.5 ± 0.9
4 Weeks	44.0 ± 0.4	44.1 ± 0.7	44.5 ± 0.8	44.3 ± 0.8
8 Weeks	43.5 ± 0.5	44.2 ± 0.7	44.1 ± 0.7	44.0 ± 0.8
MCV (fL)				
Pre	90.0 ± 1.1	90.0 ± 0.6	89.1 ± 1.1	87.8 ± 1.5
4 Weeks	89.2 ± 0.9	90.0 ± 0.9	89.3 ± 1.4	88.3 ± 1.2
8 Weeks	88.8 ± 1.0	88.8 ± 0.9	88.0 ± 1.1	88.2 ± 1.6
MCH (pg)				
Pre	30.2 ± 0.4	29.9 ± 0.3	30.2 ± 0.5	29.6 ± 0.6
4 Weeks	30.1 ± 0.3	30.0 ± 0.3	30.0 ± 0.5	29.5 ± 0.6
8 Weeks	30.3 ± 0.3	30.0 ± 0.4	30.0 ± 0.5	29.6 ± 0.7
MCHC ($g\,dL^{-1}$)				
Pre	33.5 ± 0.3	33.4 ± 0.3	33.9 ± 0.2	33.7 ± 0.2
4 Weeks	33.7 ± 0.2	33.3 ± 0.2	33.5 ± 0.2	33.4 ± 0.3
8 Weeks	34.1 ± 0.2	33.7 ± 0.2	34.0 ± 0.2	33.5 ± 0.2
RDW (%)[b]				
Pre	13.3 ± 0.1	13.3 ± 0.2	13.0 ± 0.1	13.6 ± 0.3
4 Weeks	13.3 ± 0.1	13.2 ± 0.1	13.0 ± 0.1	13.7 ± 0.4
8 Weeks	13.2 ± 0.1	13.2 ± 0.1	13.1 ± 0.2	13.8 ± 0.3
Platelets ($10^3\,\mu L^{-1}$)[b]				
Pre	232.7 ± 12.8	227.8 ± 10.2	238.8 ± 17.0	260.2 ± 12.0
4 Weeks	233.5 ± 12.6	229.1 ± 8.8	237.3 ± 14.9	259.5 ± 8.2
8 Weeks	229.9 ± 12.1	231.9 ± 8.8	242.1 ± 15.5	264.5 ± 10.9
Neutrophils (%)[b]				
Pre	51.5 ± 1.7	53.6 ± 2.7	45.1 ± 4.5	50.4 ± 2.9
4 Weeks	51.5 ± 1.9	56.5 ± 3.8	47.4 ± 3.4	47.0 ± 3.2
8 Weeks	51.9 ± 1.9	52.7 ± 2.4	50.5 ± 2.9	48.1 ± 2.7
Lymphocytes (%)				
Pre	35.8 ± 1.0	36.1 ± 2.7	42.6 ± 4.2	37.1 ± 2.5
4 Weeks	35.6 ± 1.3	32.3 ± 3.4	40.7 ± 3.1	39.8 ± 2.5
8 Weeks	34.9 ± 1.5	36.5 ± 2.5	37.6 ± 3.0	39.3 ± 2.5
Monocytes (%)[b]				
Pre	9.2 ± 0.4	7.2 ± 0.3	8.6 ± 0.6	8.5 ± 0.6
4 Weeks	8.8 ± 0.6	8.1 ± 0.7	8.1 ± 0.4	8.3 ± 0.5
8 Weeks	9.9 ± 0.7	8.0 ± 0.7	8.5 ± 0.6	8.7 ± 0.7

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Table 3. (continued)

Variable	Placebo ($n = 12$)	Higenamine ($n = 12$)	Caffeine ($n = 12$)	HCY ($n = 12$)
Eosinophils (%)				
Pre	3.0 ± 0.6	2.6 ± 0.7	3.2 ± 0.6	3.1 ± 0.4
4 Weeks	3.6 ± 0.5	2.6 ± 0.7	3.4 ± 0.8	4.0 ± 1.1
8 Weeks	2.8 ± 0.6	2.3 ± 0.4	2.9 ± 0.6	3.4 ± 0.
Basophils (%)				
Pre	0.5 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.2
4 Weeks	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.9 ± 0.2
8 Weeks	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.1

HCY: higenamine + caffeine + yohimbe; WBC: white blood cell; RBC: red blood cell; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width.
[a]Values are mean ± SEM. No other differences of statistical significance noted ($p > 0.05$).
[b]RDW ($p = 0.002$): HCY > higenamine and caffeine; platelets ($p = 0.008$): HCY > placebo and higenamine; neutrophils ($p = 0.03$): higenamine > caffeine; and monocytes ($p = 0.01$): placebo > higenamine.

Table 4. Comprehensive metabolic panel data of healthy men assigned to placebo, higenamine, caffeine, or HCY for 8 weeks.[a]

Variable	Placebo ($n = 12$)	Higenamine ($n = 12$)	Caffeine ($n = 12$)	HCY ($n = 12$)
Glucose ($mg\,dL^{-1}$)				
Pre	89.3 ± 2.5	92.7 ± 1.6	90.6 ± 2.5	89.0 ± 2.0
4 Weeks	89.9 ± 2.5	92.8 ± 1.3	88.8 ± 2.3	92.2 ± 2.4
8 Weeks	92.8 ± 1.9	91.7 ± 1.5	91.6 ± 1.2	87.4 ± 2.3
BUN ($mg\,dL^{-1}$)[b]				
Pre	16.9 ± 1.3	15.3 ± 1.0	14.7 ± 0.9	13.4 ± 0.7
4 Weeks	16.2 ± 1.0	15.7 ± 0.9	15.2 ± 1.4	13.8 ± 1.0
8 Weeks	15.3 ± 1.4	15.8 ± 1.2	14.4 ± 1.3	14.2 ± 1.0
Creatinine ($mg\,dL^{-1}$)[b]				
Pre	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
4 Weeks	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
8 Weeks	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
BUN: creatinine				
Pre	16.8 ± 1.2	14.4 ± 0.9	14.7 ± 0.7	13.8 ± 0.7
4 Weeks	16.2 ± 0.8	15.0 ± 1.3	15.0 ± 1.1	13.8 ± 0.8
8 Weeks	15.8 ± 1.4	15.1 ± 1.0	14.4 ± 1.1	14.8 ± 0.7
Sodium ($mmol\,L^{-1}$)				
Pre	141.6 ± 0.6	140.2 ± 0.4	141.2 ± 0.7	139.9 ± 0.6
4 Weeks	141.0 ± 0.7	140.4 ± 0.6	141.0 ± 0.6	140.6 ± 0.5
8 Weeks	138.4 ± 1.8	140.3 ± 0.5	140.1 ± 0.8	140.8 ± 0.7
Potassium ($mmol\,L^{-1}$)[b]				
Pre	4.8 ± 0.1	4.3 ± 0.1	4.7 ± 0.1	4.8 ± 0.1
4 Weeks	4.6 ± 0.2	4.3 ± 0.2	4.5 ± 0.1	4.9 ± 0.1
8 Weeks	4.5 ± 0.2	4.5 ± 0.1	4.5 ± 0.1	4.8 ± 0.1
Chloride ($mmol\,L^{-1}$)				
Pre	102.50.5 ±	101.6 ± 0.7	102.0 ± 1.0	100.8 ± 0.5
4 Weeks	102.9 ± 0.5	102.2 ± 0.4	101.8 ± 0.5	101.8 ± 0.4
8 Weeks	102.1 ± 0.6	101.8 ± 0.4	101.9 ± 0.6	101.3 ± 0.5

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Table 4. (continued)

Variable	Placebo ($n = 12$)	Higenamine ($n = 12$)	Caffeine ($n = 12$)	HCY ($n = 12$)
CO_2 (mmol L^{-1})[b]				
Pre	24.5 ± 0.4	24.0 ± 0.6	23.9 ± 0.4	23.4 ± 0.4
4 Weeks	25.2 ± 0.5	24.7 ± 0.4	24.0 ± 0.6	23.5 ± 0.5
8 Weeks	24.3 ± 0.4	24.8 ± 0.5	23.8 ± 0.4	23.4 ± 0.3
Calcium (mg dL^{-1})				
Pre	9.6 ± 0.1	9.7 ± 0.1	9.7 ± 0.1	9.5 ± 0.1
4 Weeks	9.5 ± 0.1	9.5 ± 0.1	9.7 ± 0.1	9.6 ± 0.1
8 Weeks	9.5 ± 0.1	9.6 ± 0.1	9.7 ± 0.1	9.6 ± 0.1
Protein (g dL^{-1})				
Pre	7.0 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.1 ± 0.1
4 Weeks	7.1 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1
8 Weeks	7.1 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.3 ± 0.1
Albumin (g dL^{-1})				
Pre	4.6 ± 0.0	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.1
4 Weeks	4.7 ± 0.0	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.1
8 Weeks	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.7 ± 0.1
Globulin (g dL^{-1})				
Pre	2.4 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.6 ± 0.1
4 Weeks	2.4 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
8 Weeks	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.6 ± 0.0
Albumin:globulin				
Pre	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.1
4 Weeks	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
8 Weeks	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.0
Bilirubin (mg dL^{-1})				
Pre	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
4 Weeks	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.7 ± 0.1
8 Weeks	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Alkaline phosphatase (IU L^{-1})				
Pre	68.5 ± 5.4	68.3 ± 4.5	68.3 ± 8.0	63.6 ± 5.1
4 Weeks	71.2 ± 5.6	69.0 ± 4.7	71.7 ± 8.9	60.4 ± 4.2
8 Weeks	72.0 ± 5.7	70.0 ± 4.5	69.2 ± 7.9	61.6 ± 4.5
AST (SGOT; IU L^{-1})				
Pre	24.4 ± 2.6	27.9 ± 2.4	26.1 ± 2.7	25.7 ± 3.5
4 Weeks	20.3 ± 1.9	25.4 ± 2.2	24.9 ± 2.6	22.4 ± 1.6
8 Weeks	23.0 ± 3.1	27.0 ± 2.8	26.1 ± 2.1	22.4 ± 1.5
ALT (SGPT; IU L^{-1})				
Pre	20.7 ± 2.8	24.3 ± 1.9	20.7 ± 3.8	22.4 ± 4.4
4 Weeks	20.5 ± 3.6	23.2 ± 1.7	20.7 ± 3.6	19.1 ± 2.4
8 Weeks	19.8 ± 3.3	24.1 ± 2.4	22.1 ± 3.7	18.3 ± 2.1
GGT (IU L^{-1})				
Pre	18.9 ± 2.4	19.9 ± 2.3	19.9 ± 3.3	31.1 ± 10.3
4 Weeks	20.2 ± 3.4	20.6 ± 3.1	21.7 ± 4.3	28.1 ± 7.4
8 Weeks	18.7 ± 2.6	20.5 ± 1.9	21.3 ± 3.7	30.2 ± 9.5

GGT: γ-glutamyl transferase; CO_2: carbon dioxide; AST: aspartate aminotransferase; SGOT: serum glutamic oxaloacetic transaminase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; HCY: higenamine + caffeine + yohimbe.

[a]Values are mean ± SEM. No other differences of statistical significance noted ($p > 0.05$).

[b]BUN ($p = 0.04$): placebo > HCY; creatinine ($p = 0.01$): higenamine > HCY; potassium ($p = 0.04$): HCY > higenamine; CO_2 ($p = 0.004$): placebo and higenamine > HCY.

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Table 5. Lipid panel data of healthy men assigned to placebo, higenamine, caffeine, or HCY for 8 weeks.[a]

Variable	Placebo ($n = 12$)	Higenamine ($n = 12$)	Caffeine ($n = 12$)	HCY ($n = 12$)
Cholesterol (mg dL^{-1})[b]				
Pre	159.3 ± 6.3	148.8 ± 10.5	177.4 ± 9.7	167.1 ± 9.3
4 Weeks	164.3 ± 7.2	149.7 ± 11.1	174.1 ± 10.1	169.5 ± 9.2
8 Weeks	166.2 ± 7.8	152.8 ± 7.4	168.8 ± 11.0	174.8 ± 8.7
Triglycerides (mg dL^{-1})[b]				
Pre	102.9 ± 11.0	72.7 ± 7.1	94.3 ± 13.2	73.2 ± 11.2
4 Weeks	116.2 ± 22.3	68.6 ± 7.8	110.9 ± 19.6	84.8 ± 14.3
8 Weeks	92.8 ± 12.0	72.5 ± 6.1	112.3 ± 21.2	77.7 ± 11.8
HDL-C (mg dL^{-1})				
Pre	49.8 ± 3.1	55.5 ± 3.0	53.3 ± 5.1	57.3 ± 3.1
4 Weeks	51.4 ± 2.9	53.9 ± 3.8	52.3 ± 5.6	56.9 ± 3.0
8 Weeks	51.3 ± 3.5	55.9 ± 3.2	50.2 ± 5.2	58.8 ± 2.9
VLDL-C (mg dL^{-1})[b]				
Pre	20.8 ± 2.2	14.7 ± 1.4	18.1 ± 2.9	14.8 ± 2.2
4 Weeks	23.3 ± 4.4	13.8 ± 1.6	22.1 ± 3.9	17.0 ± 2.6
8 Weeks	18.4 ± 2.4	14.4 ± 1.2	22.4 ± 4.3	15.6 ± 2.4
LDL-C (mg dL^{-1})[b]				
Pre	88.8 ± 5.5	78.7 ± 9.7	105.3 ± 8.1	95.1 ± 7.1
4 Weeks	89.7 ± 5.0	82.0 ± 9.5	99.8 ± 8.3	95.6 ± 6.6
8 Weeks	96.4 ± 7.1	82.5 ± 6.1	96.3 ± 8.6	100.4 ± 7.0
Total/HDL-C[b]				
Pre	3.3 ± 0.2	2.7 ± 0.2	3.6 ± 0.3	3.0 ± 0.2
4 Weeks	3.3 ± 0.2	2.9 ± 0.2	3.6 ± 0.4	3.0 ± 0.2
8 Weeks	3.4 ± 0.3	2.8 ± 0.1	3.7 ± 0.4	3.0 ± 0.2

HCY: higenamine + caffeine + yohimbe. HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol.

[a]Values are mean ± SEM. No other differences of statistical significance noted ($p > 0.05$).

[b]Cholesterol ($p = 0.01$): caffeine and HCY > higenamine; triglycerides ($p = 0.004$): placebo and caffeine > higenamine; VLDL-C ($p = 0.007$): placebo and caffeine > higenamine; LDL-C ($p = 0.01$): caffeine > higenamine; total/HDL-C ($p = 0.001$): caffeine > higenamine and HCY.

statistical significance but may require explanation was observed for GGT (Table 4). One subject assigned to HCY demonstrated very high GGT values prior to the start of supplementation—values that remained high at all collection times—greatly increasing the mean and the variability in this measure.

Subject characteristics were very similar between conditions, with no noted differences (Table 1). It should again be noted that subjects in this study were self-reported regular users of stimulants prior to enrolling in the study. It is possible that the inclusion of older, sedentary adults (either men or women), who may not be in ideal health and who may not regularly use stimulants, may produce different findings than those reported here. This possibility is also true for younger individuals (e.g. teens) who may be users of stimulant-based drinks and dietary supplements. Our use of healthy, young men exclusively may be considered a limitation of this work, as may be our relatively small sample size for each condition. Likewise, our failure to measure blood or urinary levels of higenamine[17] in our subjects' pre-, mid-, and post-intervention may be considered a limitation by some. However, it should be noted that due to the extremely short half-life of higenamine[14] (approximately 8 min; 94% of higenamine eliminated from the body within 30 min after administration), it is unlikely that values would be detected as changing significantly across time.

To our knowledge, no published human toxicology studies have been conducted using oral higenamine, alone and in combination with other agents. We are aware of one animal study to test the intravenous median lethal dose (LD_{50}) of higenamine administered to male and female mice.[18] In this study, the intravenous LD_{50} of higenamine was approximately 50 mg/kg for both male and female mice, with no gender differences noted. The authors indicated that most of the mice died of tachycardia and heavy breathing within 5 min after an intravenous dose equal to

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60 mg/kg. However, it was noted that no animals died when higenamine was administered orally at a dose of 2000 mg/kg, thus the oral LD_{50} is assumed to be greater than this value. This finding is interesting and confirms that oral higenamine is poorly absorbed in animals.[19] This topic requires further investigation using human subjects.

Feng and colleagues treated human subjects with continuous, intravenous infusion of higenamine at gradually escalating doses.[14] They determined that intravenous administration of 22.5 μg kg^{-1} was well tolerated by subjects, with only one event noted that was thought to be related to the higenamine (e.g. moderate dizziness and nausea), which was transient in nature. Assuming a 70-kg man, the noted dosage would be close to only 1.5 mg. It is possible that higher dosages may also be well tolerated, although this requires further investigation. In support of this and using an animal model, Zhang et al.[16] treated dogs with relatively high dosages of intravenous higenamine and concluded that "Higenamine can be used in pharmacological stress test with remarkable tolerability and safety even at the dosage of 500 μg kg^{-1} min^{-1} without serious adverse effect." This would translate into a dosage of approximately 35 mg for a 70-kg man. Compared with the dosage of approximately 100 mg provided to subjects in this study, this appears very low. However, as indicated in the work of Lo and Chen,[18] oral higenamine is very poorly absorbed and variable. For example, in a study using rabbits, oral bioavailability varied from approximately 3%–22%, possibly due to differences in higenamine degradation, gut metabolism, or gastrointestinal absorption.[19] In comparison, intravenous dosing is essentially 100% bioavailable. Assuming 20% bioavailability and using the estimation of 35 mg of intravenous higenamine provided by Zhang et al.,[16] oral intake as high as 175 mg may be well tolerated.

To our knowledge, this is the first study to determine the safety profile of oral higenamine when used alone and in conjunction with other commonly used lipolytic agents. We conclude that 8 weeks of supplementation with higenamine, either alone or in conjunction with caffeine and yohimbe bark extract, does not result in a statistically significant change in any of the measured outcome variables. Data are specific to a sample of healthy, young men. With the exception of one subject assigned to HCY who reported adverse effects during the initial days of using the treatment, all subjects tolerated higenamine intake well. Additional studies may be considered using subjects of both genders and of various age-groups, possibly inclusive of additional clinical and nonclinical measures (e.g. oxidative stress biomarkers, body mass, and body fat) not integrated into this study, to more fully elucidate the safety and efficacy profile of higenamine when used alone and in combination with other ingredients. Such studies may incorporate a larger sample size, more control over whether or not capsules should be ingested in a fasted state, and possibly a longer time course of treatment as compared to that used in this study.

Authors' Note

RJB was responsible for the study design, statistical analyses, and manuscript preparation. JMS and TAG were responsible for subject recruitment, data collection, data entry, and assistance with manuscript preparation. All authors read and approved the final manuscript.

Conflict of interest

RJB has been a consultant for, and/or principal investigator on research studies funded by, various dietary supplement companies. Other authors declare no competing interests.

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