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A Blend of *Chlorophytum Borivilianum* and Velvet Bean Increases Serum Growth Hormone in Exercise-Trained Men

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Abstract

Background: Several isolated ingredients have been proposed to increase growth hormone (GH) release, including *Chlorophytum borivilianum* and Velvet bean. A combination of these two ingredients has been packaged within an investigational dietary supplement. It was the purpose of the present investigation to determine the impact of acute ingestion of this supplement on circulating GH in healthy, exercise-trained men.

Methods: Fifteen men ingested the dietary supplement on two different days, separated by one week. Blood was collected from subjects before ingestion of the supplement and at 20, 40, 60, 80, 100, and 120 minutes post ingestion. GH was analyzed in serum samples using an ELISA method. Values for GH for each subject, at each collection time, were averaged over both test days and used in the main analysis.

Results: Serum GH increased over time, with higher values at 60 minutes ($1.56 \pm 0.65 \text{ ng} \cdot \text{mL}^{-1}$; $P = 0.04$; +767%), 80 minutes ($1.76 \pm 0.69 \text{ ng} \cdot \text{mL}^{-1}$; $P = 0.02$; +878%), and 100 minutes ($1.48 \pm 0.62 \text{ ng} \cdot \text{mL}^{-1}$; $P = 0.05$; +722%) compared to pre ingestion ($0.18 \pm 0.04 \text{ ng} \cdot \text{mL}^{-1}$). A great deal of subject variability existed in the area under the curve (AUC) for GH, with pooled values ranging from 0.49 to 61.2 $\text{ng} \cdot \text{mL}^{-1} \cdot 2 \text{ hr}^{-1}$.

Conclusion: Acute ingestion of an investigational dietary supplement containing the active ingredients *Chlorophytum borivilianum* and Velvet bean results in an increase in circulating GH in exercise-trained men. Additional placebo controlled investigations are needed to extend these findings. Moreover, studies are needed to determine if chronic use of such supplementation leads to favorable changes in health-related parameters associated with increased circulating GH.

Keywords: supplements, growth hormone, *Chlorophytum borivilianum*, Velvet bean, *Mucuna pruriens*

Nutrition and Metabolic Insights 2011:4 55–63

doi: [10.4137/NMI.S8127](https://doi.org/10.4137/NMI.S8127)

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Background

Growth hormone (GH) is secreted by the anterior pituitary gland regulated by two hypothalamic peptides: GH-releasing hormone (GHRH), which stimulates GH synthesis and secretion, and somatostatin, which inhibits GH release without affecting GH synthesis.^{1,2} The major influence of GH is its anabolic effect on bodily tissues, including growth, cell reproduction and regeneration. As a result, GH plays an important role during the maturation of a child through puberty, as well as helping the body to adapt to heavy physical stress (eg, resistance training). The main physiologic functions of GH include increased protein synthesis, increased lipolysis, increased collagen synthesis, increased cartilage growth, decreased glycogen synthesis and glucose utilization, and enhanced immune cell function.³

Various external factors, such as age,^{4,5} sex,⁴ sleep,^{6,7} nutritional status,^{8–10} alcohol consumption,¹¹ and exercise^{12,13} all influence GH release patterns. Moreover, a number of intravenously infused and orally consumed pharmaceutical agents have been noted to stimulate GH acutely.¹⁴

Although the consistent release of GH during sleep has been well documented,^{6,15} the release at other times of the day is intermittent, erratic, and unpredictable with no reliable or recognizable pattern.^{4,5,16,17} This pulsatile release of GH illustrates the need to measure frequent samples for accurate analysis, as well the maintenance of precise control of prior sleep and dietary intake, as these may influence circadian rhythms and GH response.

Considering the potential health and performance benefits of increased circulating GH, many dietary supplements have been produced containing combinations of ingredients purported to increase GH release. Although some isolated ingredients have been shown to be effective in this regard,^{14,18–20} many have been delivered intravenously rather than via oral ingestion, yielding little practical application to the dietary supplement market. Two ingredients that have been reported to offer health-enhancing effects, believed within the ayurvedic system to be associated with an increase in GH output, are *Chlorophytum Borivillianum*^{21,22} and Velvet bean.²³

Chlorophytum borivillianum, also known as Safed Musli, is a tropical herb with many therapeutic applications within Ayurvedic medicine. Some

of these purported benefits include enhanced immunomodulatory function,²⁴ anti-tumor, anti-mutagenic and chemomodulatory effects,²⁵ amelioration of diabetic complications,^{21,26} reduction in joint pain and rheumatoid arthritis,²¹ aphrodisiac qualities,^{26,27} and spermatogenic properties that many help with erectile dysfunction and impotency. Many of these enhanced physiological benefits may stem from its supported hypolipidemic²⁸ and antioxidant properties.²² For more information on the purported benefits of *chlorophytum borivillianum*, readers are referred to the review by Thakur et al.²¹

Velvet bean, also known as *Mucuna pruriens*, is a climbing legume that also has its heritage in Ayurvedic medicine. It has traditionally been used as treatment for degenerative disorders such as Parkinson's Disease,²⁹ and contemporary scientific trials have supported this.^{30,31} L-DOPA (L-3,4-dihydroxyphenylalanine), found within the seeds of this legume, is believed to drive the positive effects.³² L-DOPA is a naturally occurring non-protein amino acid, synthesized from L-tyrosine, and found in some foods and herbs. The most important function of L-DOPA is to act as the precursor to the neurotransmitter dopamine, as well as the catecholamines epinephrine and norepinephrine. In addition to having positive effects in patients with Parkinson's disease,^{30,31} L-DOPA has been found to stimulate GH effectively.^{14,33–37} Moreover, clinical trials have illustrated a higher effectiveness of *Mucuna* powder over synthetic L-DOPA in ameliorating Parkinson's complications³⁸ and oral consumption of *Mucuna pruriens* has elicited measurable amounts of L-DOPA in the plasma of human volunteers.³⁹

Based on the suggested evidence for *Chlorophytum borivillianum* and Velvet bean with regards to providing a stimulatory effect on GH release, an investigational dietary supplement has been developed containing a combination of these two active ingredients. It was the purpose of the present investigation to determine the impact of acute ingestion of this supplement on circulating GH concentrations in healthy, exercise-trained men.

Methods

Subjects and screening

Fifteen healthy, exercise-trained men participated in this study. All subjects completed a medical history



and physical activity questionnaire prior to being enrolled. No subject smoked cigarettes or had self reported disease of cardiovascular or metabolic origin. During the initial visit to the lab, subjects completed all paperwork and resting heart rate and blood pressure were measured (following a 10 minute quiet rest period). In addition, subjects' height, weight, waist and hip circumference, and skinfold thickness for estimation of body fat percentage (using Lange calipers, a seven-site test, and the Siri equation) was measured and recorded. Subjects were then scheduled for their other lab visits. Subject descriptive characteristics are presented in Table 1. All experimental procedures were performed in accordance with the Helsinki Declaration and The University of Memphis Human Subjects Committee approved all experimental procedures. Subjects provided verbal and written consent prior to participating in this study.

Supplement and testing

Following screening procedures, subjects reported to the lab in the morning hours (0600–0900) on two different occasions separated by one week, to undergo testing. The time of day for testing was matched for each subject. Each subject underwent two identical test days with use of the supplement (as indicated below). Average values for each blood collection time were recorded for each subject and used in data analysis. As day-to-day variation in fasting and resting GH is possible,^{8–10} as well as GH

in response to a given stimuli,^{7,11,12} we believed it would be best to have subjects undergo two identical test days and use the average values for each collection time within the analysis to better assess the acute impact of the supplement on GH response. Our noted differences in subjects' response to treatment with the supplement on the two test days confirmed our plan (see Results).

The investigational dietary supplement used in this investigation contained a proprietary blend of *Chlorophytum borivillianum* (root) and Velvet bean (bean). Although the concentration of active ingredients in each capsule was identical, two different excipients were used in manufacturing, which allowed for additional investigation into the effects of the active ingredients in terms of oral bioavailability and delivery into the system. Although this was not a chief aim of the present research, the excipient difference added a novel aspect to our work in determining the effect of the active ingredients on serum growth hormone response. Capsules for test 1 included 3% cornstarch and capsules for test 2 included 3% microcrystalline cellulose powder. Capsules for each test were from the same bottle and produced in accordance with Good Manufacturing Practices. On each test day, subjects ingested three capsules of the dietary supplement (a total of 2250 mg). The dosage provided was used in an attempt to provide adequate amounts of the active ingredients to deliver GH stimulating agents (eg, L-DOPA). No other food was allowed during the two hour post intake period. However, water was allowed *ad libitum*.

On both test days, subjects reported to the laboratory in a fasted state (≥ 10 hours). Subjects were asked not to exercise or to perform any strenuous physical activity for the 48 hours prior to each test day. Following a 10 minute rest period, a blood sample was obtained. Subjects then ingested the supplement (3 capsules) in the presence of an investigator. Additional blood samples were collected at 20, 40, 60, 80, 100, and 120 minutes following ingestion of the supplement. Subjects remained inactive in the lab during the entire two hour test period.

Blood collection and biochemistry

A total of seven venous blood samples (7 mL per draw) were taken from subjects' forearm via needle and Vacutainer[®] (pre ingestion, 20, 40, 60, 80, 100,

Table 1. Descriptive characteristics of exercise-trained men.

Variable	Value
Age (years)	23.9 ± 2.1
Height (cm)	177.0 ± 7.3
Body weight (kg)	80.3 ± 10.3
Body mass Index (kg · m ⁻²)	25.6 ± 2.6
Body fat (%)	12.6 ± 5.5
Waist (cm)	85.1 ± 6.9
Hip (cm)	101.6 ± 7.2
Waist:hip	0.84 ± 0.04
Resting heart rate (bpm)	66.4 ± 13.7
Resting systolic blood pressure (mmHg)	118.7 ± 10.1
Resting diastolic blood pressure (mmHg)	70.5 ± 9.2
Anaerobic exercise (years)	5.0 ± 3.5
Anaerobic exercise (hours · week ⁻¹)	4.3 ± 1.9
Aerobic exercise (years)	3.0 ± 3.0
Aerobic exercise (hours · week ⁻¹)	2.5 ± 1.8

Note: Values are mean ± SD.



and 120 minutes post ingestion). Blood was allowed to clot at room temperature and then processed in a refrigerated centrifuge in order to obtain serum (4 °C for 15 min at 2000 g). Serum samples were then stored at -70 °C. Assays were performed in duplicate within four weeks of sample collection. Growth hormone was determined using a solid phase sandwich enzyme linked immunosorbent assay (ELISA) method (catalog number: HG048H; Calbiotech, Spring Valley, CA). The sensitivity of this assay is 0.2 ng · mL⁻¹, while the intra- and inter-assay coefficient of variation is 5.6% and 8.0%, respectively.

Dietary intake

Subjects were required to record all food and drink consumed during the 24 hour period prior to the first test day. Records were then copied and returned to subjects so that they could duplicate this intake during the 24 hour period prior to the second test day. Diet records were analyzed for total calories, protein, carbohydrate, fat, and a variety of micronutrients (Food Processor SQL, version 9.9, ESHA Research, Salem, OR). Values for each dietary variable were averaged over the two days of recording. Dietary data are presented in Table 2.

Statistical analysis

Growth hormone data were analyzed using an analysis of variance (ANOVA) across time, with subsequent single degree of freedom contrasts performed to determine differences between pre ingestion and post ingestion collection times. Prior to this, a 2 (test day) × 7 (time) ANOVA was performed. Area under the curve (AUC) for GH was calculated for each subject as described in detail by Pruessner and colleagues.⁴⁰

Table 2. Dietary data of exercise-trained men during the 24 hour period before use of supplement.

Variable	Value
Kilocalories	2247 ± 93
Protein (g)	106 ± 8
Carbohydrate (g)	257 ± 16
Fat (g)	90 ± 5
Vitamin C (mg)	77 ± 11
Vitamin E (mg)	7 ± 1
Vitamin A (RE)	420 ± 143
Selenium (µg)	52 ± 8

Note: Values are mean ± SEM.

All analyses were performed using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC). Statistical significance was set at $P \leq 0.05$. Data are presented as mean ± SEM, except for subject descriptive characteristics (mean ± SD).

Results

With regards to the 2 (test day) × 7 (time) ANOVA, no interaction was noted ($P = 0.39$), with GH for both test days increasing over time. However, a more robust increase was noted for test 2 as compared to test 1, as evidenced by a test day effect ($P = 0.02$). A time effect was also noted ($P = 0.01$). Contrasts revealed that values were higher at 60 minutes ($P = 0.01$), 80 minutes ($P = 0.004$), and 100 minutes ($P = 0.02$) post ingestion as compared to pre ingestion. No differences were noted between pre ingestion and 20 minutes, 40 minutes, or 120 minutes post ingestion ($P > 0.05$). Data are presented in Figure 1B. When collapsing both test days and investigating the GH response over time, it was noted that GH increased in a non-statistically significant manner ($P = 0.10$). Contrasts revealed that values were higher at 60 minutes ($P = 0.04$), 80 minutes ($P = 0.02$), and 100 minutes ($P = 0.05$) post ingestion as compared to pre ingestion. No differences were noted between pre ingestion and 20 minutes, 40 minutes, or 120 minutes post ingestion ($P > 0.05$). Data are presented in Figure 1A.

As expected based on prior reports of fasting and resting GH values,⁸⁻¹⁰ as well as GH in response to various stimuli,^{7,11,12} we noted a high degree of within and between subject variability in the area under the curve (AUC) for GH (Fig. 2). In fact, when pre ingestion values were pooled for both test days, two subjects exhibited much higher pre ingestion GH values than all other subjects (1.8 ng · mL⁻¹ and 6.3 ng · mL⁻¹; compared to ≤ 0.5 ng · mL⁻¹), as well as a large increase with supplement ingestion. These subjects were subsequently removed from the ANOVA.

Discussion

Our data indicate that the investigational dietary supplement, at a dosage of three capsules, results in an increase in circulating GH in exercise-trained men. While this finding may be of interest, additional studies are needed to determine what, if any, health related effects this acute change in GH may impart. To address such a question, intervention trials involving

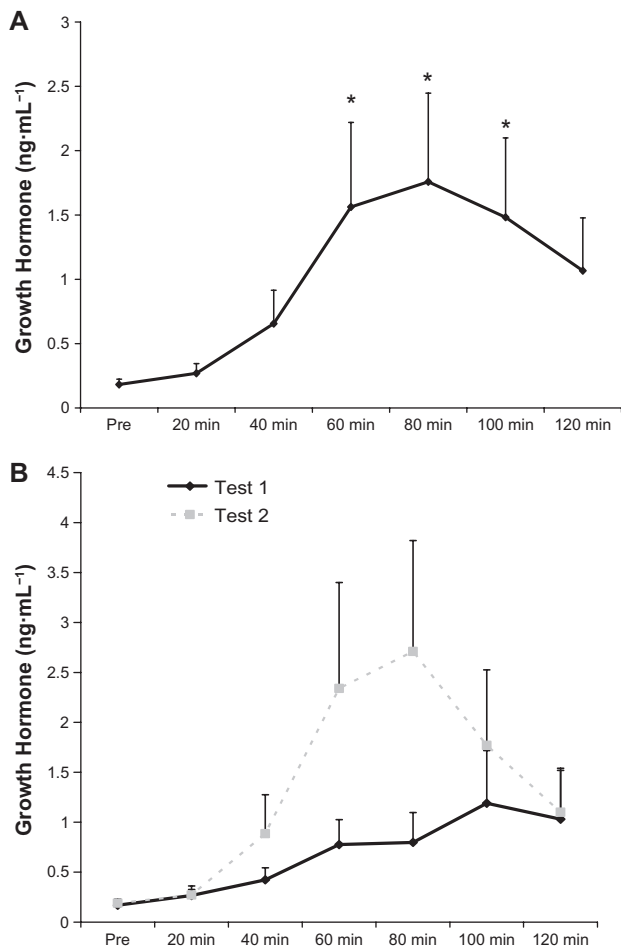


Figure 1. Serum growth hormone of 13 exercise-trained men before and during the two-hour period after oral intake of supplement. Pooled values for Tests 1 and 2 are presented in panel A. Time ($P = 0.10$); 60 min ($P = 0.04$), 80 min ($P = 0.02$), and 100 min ($P = 0.05$) greater than Pre. Independent values for Test 1 and Test 2 are presented in panel B. Test \times time ($P = 0.39$). Test ($P = 0.02$). Time ($P = 0.01$); 60 min ($P = 0.01$), 80 min ($P = 0.004$), and 100 min ($P = 0.02$) greater than Pre. **Note:** Values are mean \pm SEM.

routine use of the supplement would be needed, possibly with the inclusion of a variety of health and human performance related outcomes measures before and following chronic use of the supplement, ideally involving a double-blind, placebo controlled trial. Moreover, as the present study included both *Chlorophytum borivilianum* and Velvet bean as the combined active ingredients, future studies should seek to determine which of these agents is responsible for the effect on GH—in particular when delivered at the same dosage to the same subjects using a cross-over design.

As can be seen in Figures 1 and 2, we noted a high degree of variability between subjects with regards to GH, as well as day to day variance

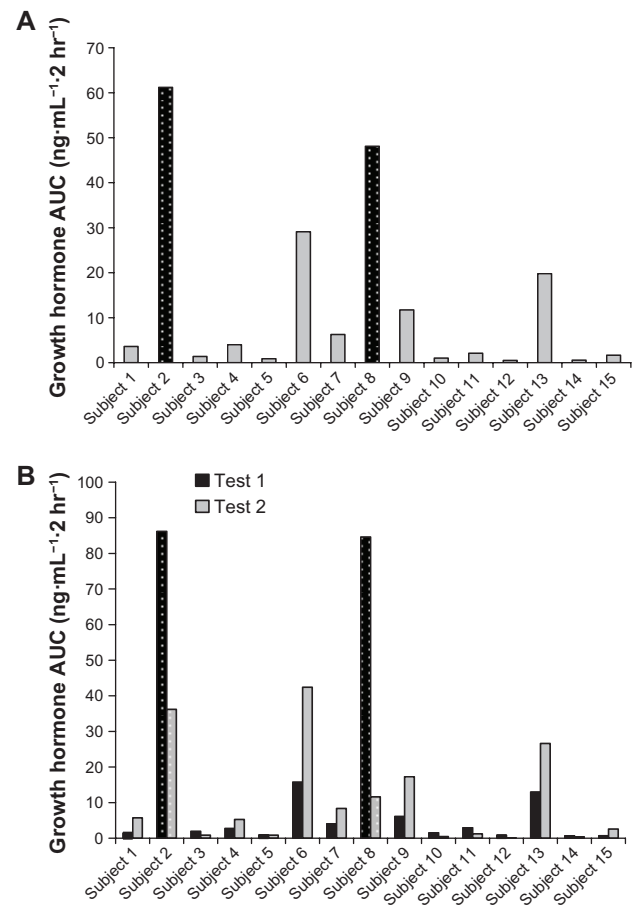


Figure 2. Serum growth hormone AUC of 15 exercise-trained men during the two-hour period after oral intake of supplement. Pooled values for Tests 1 and 2 are presented in panel A. Subject 2 (Pre GH value = $6.3 \text{ ng} \cdot \text{mL}^{-1}$) and Subject 8 (Pre GH value = $1.8 \text{ ng} \cdot \text{mL}^{-1}$) were excluded from the time analysis due to high Pre GH levels. All other subjects had Pre GH levels $\leq 0.5 \text{ ng} \cdot \text{mL}^{-1}$ (Pre GH range: $0.02\text{--}0.5 \text{ ng} \cdot \text{mL}^{-1}$). Independent values for Test 1 and Test 2 are presented in panel B.

within subjects (which may be partly related to the difference in excipients used). This between subject variation is an important consideration when attempting to extrapolate our pooled data to individuals outside of our subject sample, as not all individuals may respond to the same extent to treatment with the supplement. Clearly, this variability is expected and is well-described with the use of oral dietary supplements.^{41,42} With this understanding, when considering the pooled data, it is apparent that the GH response over the two hour post ingestion period is significant, in particular at the 80 minute post ingestion time, when GH reached a peak of $1.76 \pm 0.69 \text{ ng} \cdot \text{mL}^{-1}$.

Lin et al¹⁴ measured the increase in GH following the administration of four metabolized stimuli, delivered either intravenously (insulin and adrenocorticotrophic



hormone [ACTH]), intramuscularly (glucagon), or orally (L-DOPA). The results for GH increase were greater than our own; however, it should be noted that a high degree of variability also existed. L-DOPA specifically caused an augmentation in GH to approximately $15 \text{ ng} \cdot \text{mL}^{-1}$, following a 500 mg oral dose. Similar findings were noted by Kansal and coworkers using the same dosage of L-DOPA.³⁷ Therefore, despite an approximate 9 fold increase from our subjects' pre ingestion value of $0.18 \pm 0.04 \text{ ng} \cdot \text{mL}^{-1}$, it is unknown what an absolute peak GH value of $<2 \text{ ng} \cdot \text{mL}^{-1}$ translates to from a physiological point of view. However, it is important to note that the actual L-DOPA amount delivered in our 3 capsule serving of this investigational formulation was lower than the amount provided in the work of Lin et al¹⁴ and Kansal et al,³⁷ due to a modification in the standardization procedure for the *Mucuna pruriens*. Additional study with a formulation that provides a standardized amount of L-DOPA that more closely mimics that provided in the work of Lin et al¹⁴ is now being planned. More work is needed to determine what, if any, physiologic benefit would be gained by transiently and mildly increasing circulating GH with the use of oral dietary supplements.

With regards to oral dietary supplements used to induce an increase in GH release in human subjects, a number of investigations have utilized various amino acids or their isoforms. Because amino acids are the building blocks of proteins, they are a logical starting point for increasing growth. Despite some evidence supporting the ability of amino acids to stimulate GH, it is certainly not conclusive and supported by the entire scientific community.⁴³ Arginine has shown some promise in relation to GH release,⁴⁴ with evidence for GH stimulation in human subjects known for many years.¹⁸ Orally administered arginine aspartate ($250 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for one week in healthy men, increased the slow wave sleep related GH secretion by at least 24% in a sample of five subjects.⁴⁵ A combination of acetyl-L-carnitine and L-ornithine have been hypothesized to stimulate GH, noted in a case-study report;⁴⁶ however, we are unaware of clinically controlled investigations with regards to these agents, either individually or in combination. A proprietary combination of L-arginine, L-ornithine, and the branched chain amino acids (L-leucine, L-isoleucine, and L-valine) elicited a higher GH area under the curve compared to placebo

in 10 moderately trained male athletes.⁴⁷ L-tryptophan has been shown to induce a slight rise in plasma GH in men and women at $70 \text{ mg} \cdot \text{kg}^{-1}$.⁴⁸ Finally, although gamma-aminobutyric acid is technically an amino acid, it is rarely referred to as such because it is not incorporated in biological proteins ("alpha-amino acids"). Nonetheless, gamma-aminobutyric acid has been shown to elevate resting and post-exercise GH in resistance trained men consuming a dosage of 3 g.⁴⁹

Agents outside of amino acids are less frequently studied with regards to GH release; however, intravenous infusion of cytidine diphosphate choline has been shown to significantly raise serum GH above basal levels⁵⁰ in a sample of six healthy men. Moreover, alpha-glycerol-phosphorylcholine, a putative acetylcholine precursor, has also been reported to elicit GH secretion in young and old human volunteers.⁵¹ Finally, as alluded to earlier, Lin et al¹⁴ illustrated GH secreting efficacy with intravenous insulin and ACTH, as well as intramuscular glucagon.

With regards to the active ingredients contained within the tested supplement, to our knowledge, currently there exist no reports pertaining to the efficacy of *chlorophytum borivilianum* or velvet bean *directly* stimulating GH in animals or humans. However, a number of investigations have reported on the efficacy of L-DOPA (a constituent of velvet bean) on stimulating GH.^{14,34,37,52-55} Considering this, our noted increase in GH is not surprising. However, future work is needed, ideally using a modified form of the supplement which is standardized for a higher concentration of L-DOPA (possibly using cellulose as the preferred excipient), in order to extend our findings.

Within the context of the above discussion, it is important to note the limitations of this work. First, we did not include a placebo condition in the analysis. Although fasting GH concentrations may fluctuate with extended fasting,⁸ it has been shown that over an acute fasting period, as done in the present study, GH remains relatively stable.^{8,10} Therefore, while we cannot be certain of this due to our lack of a placebo control, our noted increase in GH across time in the present study is very likely due to intake of the supplement, rather than other factors such as fasting or diurnal variation. Despite this, future studies should include a placebo condition for comparison. Second, we only included a two hour



post ingestion measurement period. Therefore, while it appears as though GH was on the decline at the 120 minute collection time (Fig. 1), we cannot confidently comment on the response beyond this two hour period. Furthermore, we cannot conclude on the long-term health and performance effects that this transient increase in GH might provide. We acknowledge that well-controlled investigations are needed to determine the chronic effect of this supplement on health and performance related parameters thought to be influenced by GH (eg, muscle mass, muscular strength). Third, we used healthy, young men exclusively as subjects in the present investigation. Hence, results should not be generalized to those with known disease, older individuals, or women, as these individuals may not respond in the same manner as our subjects did. Fourth, our tests were performed outside of the context of an acute exercise bout. It is possible that differing results may have been observed if the supplement was taken prior to an exercise bout, as exercise is also known to stimulate GH release.¹³ Future study is needed to determine the combined impact of the supplement and acute exercise on GH release, as well as health related outcomes owing to such changes in GH over time.

Conclusion

In conclusion, we report that an investigational dietary supplement containing the active ingredients *Chlorophytum borivilianum* and Velvet bean resulted in an increase in serum GH when ingested by young and healthy exercise-trained men. This treatment was well-tolerated and should be considered safe, as evidenced by prior work using both *Chlorophytum borivilianum*⁵⁶ and Velvet bean,^{39,57,58} which has indicated no toxicity or adverse outcomes with use. Additional studies are needed to determine if the chronic use of this supplement, or others like it, would result in meaningful changes in health related parameters associated with increased circulating GH.

Acknowledgments

Funding for this work was provided in part by USPlabs, LLC and the University of Memphis.

Competing Interest

Financial support for this work was provided in part by USPlabs, LLC. None of the authors have a financial

interest in this company. RJB has received research funding or acted as consultant to other nutraceutical and dietary supplement companies. All other authors declare no competing interests.

Authors' Contributions

RJA, REC, and CGM were responsible for data collection, blood collection and processing, data entry, and assistance with manuscript preparation. RJB was responsible for the study design, biochemical work, statistical analyses, and manuscript preparation. All authors read and approved of the final manuscript.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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