10761 King William Drive | Dallas, TX 75220
Phone 469 484 1927 | Fax 469 484 1930 | www.USPlabsDirect.com

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Quyen Tien
Division of Enforcement
Office of Compliance (HFS-608)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740-3835

Re: Warning Letter No. 285519 (April 24, 2012)

Dear Mr. Tien:

This letter responds to the warning letter from Michael W. Roosevelt (the Roosevelt letter) dated April 24, 2012, regarding the marketing of our dietary supplements that contain the dietary ingredient, 1,3-dimethylamylamine HCl (DMAA). The Roosevelt letter states that (1) FDA knows of no information demonstrating that DMAA is a dietary ingredient, (2) assuming DMAA is a dietary ingredient, it is a new dietary ingredient (NDI) for which a notification has not been submitted, and (3) FDA knows of no evidence demonstrating the safety of DMAA as a dietary ingredient. This letter and its appendices demonstrate that DMAA complies with the applicable requirements of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for a lawful dietary ingredient and does not present a significant or unreasonable risk of illness or injury to consumers under the conditions of use recommended or suggested in the labeling.

I. DMAA is a Dietary Ingredient

The intent of Congress to authorize the marketing of a wide range of dietary ingredients is evident in its broad and comprehensive definitions of a "dietary supplement" and a "dietary ingredient." Section 201(ff)(1) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) defines a dietary supplement as:

> a product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (A) a vitamin; (B) a mineral; (C) an herb or other botanical; (D) an amino acid; (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (F) a concentrate, metabolite, constituent, extract or combination of any ingredient described in clause (A), (B), (C), (D), or (E).[1]

The dietary substance involved here, DMAA, satisfies the statutory definition of a dietary ingredient in two ways: (1) under Sections 201(ff)(1)(C) and (F), as a constituent of a botanical -- the geranium plant -- and (2) under Section 201(ff)(1)(E), as a dietary substance for use to supplement the diet.

A. Geranium is a Botanical

The FD&C Act does not define the word "botanical." According to the *Concise Oxford English Dictionary*, the word "botanical" means "a substance obtained from a plant and used as an additive."[2] Geranium (*Pelargonium graveolens*), as described in *The Oxford Companion to Food*, is a popular garden plant, i.e., a botanical.[3] DMAA is a substance obtained

[1] FD&C Act § 201(ff)(1).

[2] Concise Oxford English Dictionary 162 10th ed. (2002) (Appendix 1).

[3] Alan Davidson, The Oxford Companion to Food 336 (Tom Jaine ed., 2d ed. 2006) (Appendix 2).

from the geranium plant. It is found in the oil obtained from the steam distillation of or extraction from geranium stems and leaves and is used as an additive.

B. DMAA is a Constituent of Geranium

Three independent scientific studies have verified the presence of DMAA as a constituent in geranium stems and leaves.

1. The Ping Study

The first of these studies was conducted by Ping et al., and was published in the *Journal of Guizhou Institute of Technology* in 1996.[4] The Ping study performed capillary gas/chromatography-mass spectrometry analysis of geranium oil, which they obtained after they procured fresh air-dried stems and leaves from the Rongjiang region of the Guizhou province in China. The report of the Ping study identified DMAA as a constituent of the geranium plant grown in that region.

Critics of the Ping study erroneously cite published papers reporting studies that fail to find DMAA in geranium oil. The primary limitations of these studies are that (1) they were designed to detect major constituents of geranium, but not constituents that appear in small amounts (none of these studies accounted for all constituents of the geranium they analyzed), (2) the analytical methods they used were not well suited for detecting DMAA in a complex matrix like geranium oil that contains hundreds of constituents, and (3) they did not use geranium oil from the Rongjian region of the Guizhou province in China, or from China at all, despite the fact that geranium oil from China is different from other types of geranium oil.

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[4] Ping Z, Jun Q, and Qing L, *A Study on the Chemical Constituents of Geranium Oil, J. of Guizhou Institute of Technology*, volume 25, at 82-85 (February 1996) (the Ping Study) (Appendix 3). A copy of the article as published in Chinese is attached in Appendix 4.

2. The Intertek Study

Intertek Health Sciences International, a leading scientific consulting firm that specializes in food science, has conducted a laboratory analytical study that documents in detail the presence of DMAA in geranium.[5] In its assessment of the results of the Intertek study, Cantox definitively concluded that DMAA exists in geranium oil and geranium plant tissues.[6]

3. The Simone Study

A third study, by Dr. Paul Simone of the University of Memphis, to determine whether DMAA is present in the geranium plant has just been completed, and an abstract of the results of the study is now available.[7] The study first retested a sample that had been tested by Intertek, and confirmed that it contained DMAA. They then tested samples from three geographically different areas. One sample had detectable DMAA and two did not. Because the presence and level of DMAA in geranium varies in different geographical areas, this is not a surprising result.

As these three independent scientific studies show, DMAA is a constituent of geranium, which makes DMAA a dietary ingredient under Section 201(ff)(1)(C) and (F).

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[5] Intertek Health Sciences International, *DMAA: Review of Safety Data and Occurrence in the Geranium Plan and Its Essential Oil* (February 6, 2012) (Appendix 5); Barry Lynch, *Memorandum* (September 6, 2011) (Appendix 6)

[6] Intertek Health Sciences International, *supra* n. 5, at 13.

[7] Fleming HL, Ranaivo PL, and Simone PS, *Analysis of 1,3- and 1-4 Dimethylpentylamine in Geranium Herb by LC-MS/MS* (Abstract) (May 2012) (Appendix 7).

C. DMAA is a Dietary Substance for Use to Supplement the Diet

DMAA is a dietary ingredient under Section 201(ff)(1)(E) because it is a "dietary substance for use by man to supplement the diet by increasing the total dietary intake." DMAA, as part of the geranium plant, has been consumed as a dietary substance for over a century.[8] Geranium leaves are infused for use as a tea and are added to desserts and confections.[9] DMAA has been consumed in geranium oil that is used as a flavoring agent.[10] Even FDA has recognized the use of geranium ingredients in food by classifying geranium as generally recognized as safe (GRAS) for use in food.[11] And DMAA is marketed specifically to increase the dietary intake of DMAA, the necessary prerequisite to qualifying as a dietary ingredient under Section 201(ff)(1)(E). As such, DMAA qualifies as a dietary ingredient under Section 201(ff)(1)(E), in addition to qualifying as a dietary ingredient under Section 201(ff)(1) (C) and (F).

II. Both Natural and Synthetic DMAA Qualify as a Dietary Ingredient

The Roosevelt letter states that synthetic DMAA is not a dietary ingredient. As explained below, the position taken in the Roosevelt letter that a synthetic ingredient that is chemically identical to a dietary ingredient found in nature cannot be a dietary ingredient is contrary to the statutory language and the intent of Congress in enacting the Dietary Supplement Health and Education Act (DSHEA).

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[8] The Oxford Companion to Food, *supra* n. 3, at 336.

[9] Deni Brown, Encyclopedia of Herbs & Their Uses 324 (1995) (Appendix 8).

[10] The Oxford Companion to Food, *supra* n. 3, at 336.

[11] 21 C.F.R. §§ 182.10 and 182.20.

A. FDA Has Long Determined that There is No Difference Between a Natural Substance and Its Synthetic Counterpart

Synthetic ingredients that are chemically identical to naturally derived ingredients, such as DMAA, may be marketed under both Sections 201(ff)(1)(C) and (F) and Section 201(ff)(1)(E) of the FD&C Act. No distinction is made in the statute between natural and synthetic dietary substances, and FDA has never previously established such a distinction. If such a distinction were to be established in the future, it must be made through notice-and-comment rulemaking under the Administrative Procedure Act,[12] not in a letter.

FDA has a long history of recognizing that synthetic ingredients identical to natural ingredients should be treated the same. FDA's nutrition labeling regulation states that a food is deemed misbranded if its labeling states or implies "That a natural vitamin in a food is superior to an added or synthetic vitamin."[13] This prohibition dates back to the late 1960s, when the agency vigorously defended its position on this issue during two years of public hearings on special dietary food regulations.[14] At the end of that formal rulemaking process, FDA concluded that "There is no nutritional difference between a vitamin provided by a synthetic source and the same vitamin provided by a natural source . . . ."[15]

As recently as the late 1990s, FDA reaffirmed the validity of the prohibition on distinguishing between natural and synthetic ingredients, stating that it is "aware of nothing that establishes that a claim of difference between the natural and synthetic version of the same form

[12] 5 U.S.C. § 553.

[13] 21 C.F.R. § 101.9(k)(4).

[14] These hearings took place between 1968 and 1970. *See* 38 Fed. Reg. 2143, 2147, 2150 (January 19, 1973).

[15] *Id.* at 2147.

of a nutrient is not misleading."[16] Denying the validity of synthetic botanical extracts would suggest that FDA now views a material distinction between synthetic and natural versions of identical ingredients. This radical shift makes no sense in light of the agency's consistent historical policy.

B. FDA Has Approved Both the Natural and the Synthetic Sources of Essential Vitamins

Several concrete examples illustrate that FDA recognizes the equivalence of naturally extracted sources and synthetic counterparts of dietary ingredients. FDA has affirmed as GRAS both natural and synthetic riboflavin,[17] vitamin A,[18] and vitamin D.[19] FDA approved the food additive Vitamin D_3 in both natural and synthetic forms.[20] And FDA has acknowledged new dietary ingredient (NDI) notifications for nature-identical synthetic botanical ingredients without objection in the past. For example, in March 2001 FDA determined that "Roche synthetic zeaxanthin is identical to natural zeaxanthin."[21]

C. Distinguishing Between Natural and Synthetic Versions of Dietary Substances is Inconsistent with FDA Policy on Genetically Engineered Food

As evinced in the domain of genetically engineered food, FDA has long maintained that the method of a product's manufacture is not a material fact unless it renders a

[16] 62 Fed. Reg. 49826, 49841 (September 23, 1997).

[17] 21 C.F.R. § 184.1695(a).

[18] 21 C.F.R. § 184.1930(a).

[19] 21 C.F.R. § 184.1950(a).

[20] 21 C.F.R. § 172.380(a).

[21] Letter from A. Davidovich, Roche Vitamins, to Office of Nutritional Products, Labeling, and Dietary Supplements, CFSAN, submitting Rpt. 96, Docket No. FDA-1995-S-0039 (March 22, 2001).

substantive change in the product itself.[22] With regard to genetically engineered foods, FDA has repeatedly concluded that a genetically engineered food does not differ in any material respect from a traditional food. FDA has therefore concluded that it lacks the statutory authority to require any special labeling for genetically engineered food. The agency's position has been upheld in court.[23]

Here, synthetic DMAA is indistinguishable from naturally occurring DMAA. As discussed above, DSHEA draws no distinction between two chemically identical dietary ingredients. FDA's conclusion to the contrary is unsupported by the statutory text, is contrary to Congress's goal that consumers have access to a wide variety of dietary supplements, is contrary to the agency's position on genetically engineered foods, and is not grounded in any scientific or public health basis. Indeed, if FDA were to adopt the position espoused in the Roosevelt letter the agency would inherently reopen its longstanding policy on the approval and labeling of genetically engineered food.

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[22] 57 Fed. Reg. 22984, 22991 (May 29, 1992). The agency reaffirmed this policy in developing its 2001 guidance on genetically engineered foods. FDA, *Draft Guidance for Industry: Voluntary Labeling Indicating Whether Foods Have or Have Not Been Developed Using Bioengineering* (January 2001) (stating that "The agency is still not aware of any data or other information that would form a basis for concluding that the fact that a food or its ingredients was produced using bioengineering is a material fact that must be disclosed under sections 403(a) and 201(n) of the act.").

[23] *Alliance for Bio-Integrity v. Shalala*, 116 F. Supp. 2d 166, 178-78 (D.D.C 2000) (concluding that FDA's assertion that it lacks the statutory authority to require special labeling for genetically engineered foods was not arbitrary or capricious); *Stauber v. Shalala*, 895 F. Supp. 1178 (W.D. Wis. 1995) (holding that FDA did not act arbitrarily and capriciously in not requiring the labeling of dairy products derived from cows treated with bovine somatotropin).

D. FDA Has Determined that Synthetic Dietary Substances are Dietary Ingredients

The breadth of Section 201(ff)(1)(E) demonstrates Congress's goal of securing consumer access to a wide variety of safe and beneficial dietary supplements and dietary ingredients.[24] The only prerequisite to being considered a dietary ingredient under Section 201(ff)(1)(E) is that the dietary ingredient be used to supplement the diet.

FDA has previously acknowledged the breadth of Section 201(ff)(1)(E) in the preamble to its regulation on requirements for nutrient content claims, health claims, and statements of nutritional support for dietary supplements.[25] There, the agency stated in a Federal Register preamble -- which constitutes a formal agency advisory opinion[26] -- that a substance such as CoQ10 -- which is commonly synthesized -- falls within the broad range of dietary ingredients that Congress contemplated.[27] FDA's acceptance of a broad range of dietary ingredients is consistent with the fact that neither the language of DSHEA nor its legislative history reveals any congressional intent to exclude synthetic versions of natural botanical extracts from the definition of a "dietary ingredient."

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[24] "Legislative action that protects the right of access of consumers to safe dietary supplements is necessary in order to promote wellness." DSHEA, Pub. L. No. 103-417, § 2(15)(A), 108 Stat. 4325, 4326 (1994).

[25] 62 Fed. Reg. 49859, 49860 (September 23, 1997).

[26] 21 C.F.R. 10.85(d)(1).

[27] *Id.* (quoting from the legislative history of "other nutritional substances" -- a precursor to "dietary ingredients" -- statements that numerous ingredients not traditionally or historically viewed as food substances would be included, such as primrose oil, black currant seed oil, amino acids, and hydrogen peroxide).

E. Common Use in Food is not a Statutory Requirement for a Dietary Ingredient

The Roosevelt letter suggests that synthetic DMAA may not be considered a dietary ingredient under Section 201(ff)(1)(E) because synthetic DMAA has not been commonly used as a food or drink. But neither the text of Section 201(ff)(1)(E) nor any legislative history contains or implies any such historical-use requirement. Section 201(ff)(1)(E) requires only one prerequisite for a dietary ingredient: that the dietary ingredient be used to supplement the diet. Where Congress intended to impose a historical-use requirement in DSHEA, it did so expressly.[28] The absence of a historical-use requirement in Section 201(ff)(1)(E) makes clear that Congress intended to apply no such limitation on the dietary ingredients encompassed by Subparagraph (E). Section 201(ff)(1)(E) contains only a forward-looking assessment. FDA's attempt to read a historical-use requirement into Subparagraph (E) is contrary to a fundamental canon of statutory construction: "*expressio unius est exclusio alterius*" (the inclusion of one is the exclusion of others). Finally, even if such a requirement had been included in the statute, the use of geranium in the food supply for more than a hundred years would satisfy it.[29]

F. Distinguishing Between Natural Dietary Substances and their Synthetic Counterparts Represents Detrimental Public Policy

As a practical matter, if FDA's interpretation of Section 201(ff)(1)(E) were correct, and synthetic botanicals were disqualified from being "dietary ingredients" altogether, such an interpretation would have a serious unintended public consequences. Manufacturers typically have more control over synthetic processes than over natural extraction processes, and

[28] *E.g.*, FD&C Act §§ 413(a)(1), (d).

[29] See Part I(C) of this letter.

this control can yield tangible safety and quality benefits for consumers. Synthetic processing can eliminate potentially harmful variables such as pesticide contamination, the presence of foreign materials, and the uptake of heavy metals and toxins from the soil. Chemical synthesis also ensures greater consistency in output quality, as variations in climate or geographic region no longer pose concerns.

Discouraging the industry's use of synthetic processing can also negatively affect the environment. If a chemical component of a plant has beneficial health effects, but turns out to be difficult to extract from its natural source on a commercial scale, the position taken in the Roosevelt letter would force industry to destroy botanicals on a large scale in order to obtain a commercial supply. The result would be to require a manufacturer to produce it in an unsustainable or environmentally irresponsible manner.[30] This simply is not what Congress intended.

III. Because DMAA Is Found In Food, No NDI Notification Is Required

The Roosevelt letter asserts that, even if DMAA is a "dietary ingredient" -- which, for the reasons explained above, it is -- DMAA must be the subject of an NDI notification

[30] The detrimental impact that the agency's policy would have on the environment has been demonstrated previously with regard to taxol, which is derived from the bark of the Pacific yew tree. The production of small amounts of natural taxol required use of large numbers of Pacific yews. Congress became concerned with the detrimental effect that the development of taxol had on the Pacific yew and therefore passed the Pacific Yew Act. Pub. L. 102-335, 106 Stat. 859 (1992). In the Act, Congress specifically stated that "appropriate management guidelines must be implemented promptly in order to prevent any wasting of the Pacific yew . . . while successful and affordable alternative methods of manufacturing taxol are being developed." By the end of 1994, Bristol-Myers Squibb discontinued selling taxol derived from the Pacific yew, and began selling only taxol that was developed through a semisynthetic alternative method of manufacture. *New Version of Taxol is Approved by F.D.A.*, N.Y. Times, December 13, 1994, *available at* http://www.nytimes.com/1994/12/13/science/new-version-of-taxol-is-approved-by-fda.html.

under Section 413(a)(2) of the FD&C Act. The Roosevelt letter's reliance on Section 413(a)(2) is misplaced.

Section 413(d) of the FD&C Act defines an NDI as a dietary ingredient that was not marketed in the United States before October 15, 1994, and which does not include any dietary ingredient that was marketed in the United States before that date.[31] Under Section 413(a), a dietary supplement containing an NDI will be deemed adulterated unless it meets one of the following two requirements: (1) it contains only dietary ingredients that have been present in the food supply as an article used for food in a form in which the food has not been chemically altered, or (2) notification is submitted to FDA by the manufacturer or distributor of the dietary ingredient or dietary supplement that there is a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions of use recommended or suggested in its labeling, will reasonably be expected to be safe.[32] An NDI that satisfies Section 413(a)(1) is exempt from the NDI notification requirement found in Section 413(a)(2).

Under Section 413(d), DMAA is an NDI because it was not marketed in the United States prior to October 15, 1994. But as explained in Part I(C) of this letter, DMAA has been present in the international food supply for over a century in a variety of ways and synthetic DMAA is chemically identical to naturally occurring DMAA. The presence of DMAA in the international food supply is sufficient under Section 413(a)(1) because Congress did not limit the geographic scope of the food supply criterion found in that provision. Where Congress wanted

[31] *Id.* § 413(d).

[32] *Id.* § 413(a).

to place a geographic limitation in the statute, it did so expressly.[33] As such, DMAA has been present in the food supply and is extracted from geranium without chemical alteration. Thus, DMAA falls under Section 413(a)(1), and not Section 403(a)(2). We are therefore not required to submit an NDI notification to FDA for the DMAA contained in our dietary supplements.

IV. Substantial Data and Analyses Establish A Reasonable Assurance That DMAA Does Not Present A Significant Or Unreasonable Risk Of Illness Or Injury

As FDA recently stated, the DMAA letters were sent only because the recipients of the letters, according to FDA, were required to but "had not submitted NDI notifications for their DMAA supplements."[34] The Roosevelt letter nonetheless states that "To the best of FDA's knowledge, there is no history of use or other evidence of safety establishing that [DMAA] will reasonably be expected to be safe as a dietary ingredient." The letter speculates as to the potential for adverse effects on human health, but references no scientific or medical evidence that DMAA is unsafe or causes harm when consumed in accordance with the labeled Directions for Use and Warnings. The published literature, together with the evaluations conducted by highly respected experts in toxicology and pharmacology, demonstrate the safety of DMAA when used as directed.

Congress's statutory presumption of the safety of dietary ingredients is evident in its explicit pronouncement that "dietary supplements are safe within a broad range of intake, and

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[33] *E.g.*, FD&C Act Section 413(d) (defining an NDI based on its presence in the United States market prior to October 15, 1994).

[34] Elaine Watson, *AHPA: If DMAA is in geranium, synthesized version is lawful dietary ingredient* (April 30, 2012), *available at* http://www.nutraingredients-usa.com/Regulation/AHPA-If-DMAA-is-in-geranium-synthesized-version-is-a-lawful-dietary-ingredient.

safety problems with the supplements are relatively rare."[35] All dietary supplements and dietary ingredients, new and old, are subject to an adulteration provision prohibiting products that present a "significant or unreasonable risk of illness or injury."[36] For a dietary supplement containing an NDI, there must exist adequate information "to provide a reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury."[37] In the case of DMAA, there is no question that adequate information provides reasonable assurance that DMAA does not present a significant or unreasonable risk of illness or injury.

A. Several Human Studies Support the Safety of DMAA

Seven clinical studies have been conducted to evaluate the safety of DMAA. These studies evaluated the safety of DMAA after single-dose administration and repeat-dose administration over periods of 2, 8, and 10 weeks. Each study has shown that, although DMAA can produce a mild and transient increase in systolic blood pressure, no other safety variables were affected. All seven studies, which are described below and appended to this response, confirm that DMAA is safe when used according to the labeled Directions for Use and Warnings.

- Bloomer et al. (2011a), *Effects of 1,3-Dimethylamylamine and Caffeine Alone or in Combination on Heart Rate and Blood Pressure in Healthy Men and Women*: This study investigated the addition of DMAA and caffeine on resting hemodynamic properties and endogenous sympathetic catecholamine (epinephrine and norepinephrine) levels of volunteers (5 female/5male) for up to 2 hours after dosing. Results: Acute ingestion of DMAA alone and in

[35] Pub. L. No. 103-417, § 2(14).

[36] FD&C Act § 402(f)(1)(A).

[37] FD&C Act § 402(f)(1)(B).

combination with caffeine results in an increase in systolic blood pressure, diastolic blood pressure, and rate pressure product without an increase in heart rate. (Appendix 9.)

- Bloomer et al. (2011b), *Effect of Caffeine and 1,3- Dimethylamylamine on Exercise Performance and Blood Markers of Lipolysis and Oxidative Stress in Trained Men and Women*: Twelve exercise-trained subjects ingested placebo, caffeine, or caffeine plus DMAA 60 minutes before running 10km. Results: A combination of 1mg DMAA/kg and 4mg caffeine/kg, a dose level approximately equivalent to the maximum product label dose, did not significant change physical performance, level of exertion, subject mood or vigor, heart rate, or blood pressure endpoints, compared to placebo, following a very strenuous physical activity. (Appendix 10.)

- Farney et al. (2012), *Hemodynamic and Hematologic Profile of Healthy Adults Ingesting Dietary Supplements Containing 1, 3- Dimethylamylamine and Caffeine*: This study investigated hemodynamic, hematological, and clinical chemistry effects of Jack3d after single and 14-day dosing in seven health adult males. Results: After dosing on days 1 and 14, systolic blood pressure increased (122-12 mm Hg) over pre-ingestion values (109 mm Hg) beginning at 30 minutes. There were not significant differences in acute changes in heart rate, diastolic pressure, or rate pressure product on days 1 or 14, After 14 days of dosing, no significant changes in hemodynamic endpoints compared to day 1 were reported. Fourteen days of dosing did not affect results of blood tests, including complete blood counts and lipid and metabolic panels. (Appendix 11.)

- Farney et al. (2012), *Hemodynamic and Hematologic Profile of Health Adults Ingesting Dietary Supplements Containing 1, 3- Dimethylamylamine and Caffeine*: This study investigated hemodynamic, hematological, and clinical chemistry effects of OxyElite Pro after singe and 14-day dosing in four healthy adult males and two females. Results: After dosing on day 1, systolic blood pressure increased (116-119 mm Hg) over pre-ingestion values (103 mm Hg) beginning at 60 minutes. There were no significant differences in acute change in systolic pressure on day 14, or in heart rate, diastolic pressure, or rate pressure product on days 1 or 14, After 14 days of dosing, no significant changes in hemodynamic endpoints compared to day 1 were report. Fourteen days of dosing did not affect results of blood tests, including complete blood counts and lipid and metabolic panels. (Appendix 11.)

- McCarthy et al. (2012a), *A Finished Dietary Supplement Stimulates Lipolysis and Metabolic Rate in Young Men and Women*: This study examined the effect of a single dose of OxyElite Pro on hemodynamics of healthy adults for up to two hours after treatment. Six males and 6 females were administered

two capsules of OxyElite Pro or placebo on two separate days in a cross-over study design. Results: An increase in heart rate of 8-11 beats/min was reported in the treated group beginning at 60 minutes. Systolic blood pressure increased (112-118 mm Hg) in the treated groups, compared to placebo (101-104 mm Hg) beginning at 30 minutes after dosing. The rate pressure product increased in the treated group at 60 minutes after dosing. There was no increase in diastolic pressure. (Appendix 12.)

- McCarthy et al. (2012b), *Biochemical and Anthropometric Effects of a Weight Loss Dietary Supplement in Health Men and Women*: This study examined the effect of an 8-week exposure of OxyElite Pro on hemodynamic, hematological, and clinical chemistry end points. Groups of 16 health adult males and females consumed 1-2 capsules of OxyElite Pro or two placebo capsules daily for 8 weeks. Results: In the treated group, resting heart was slightly, but statistically significantly, higher (69.4 BPM) at the end of the study compared to the beginning (63.3 BPM), but were not different from placebo control values. There were no differences in systolic or diastolic blood pressure between treatment groups. There were also no clinically relevant differences between treatment groups across time in hematology, lipid, or metabolic panel endpoints. (Appendix 13.)

- Whitehead et al. (2012), *Impact of a Dietary Supplement Containing 1,3-Dimethylamylamine on Blood Pressure and Bloodborne Markers of Health: a 10-week Intervention Study*: This study examined the effect of a 10-week exposure of Jack3d on hemodynamic, hematological, and clinical chemistry endpoints in groups of 12 to 13 healthy adult males. Results: Ten weeks of Jack3d use resulted in reported heart rate and systolic and diastolic blood pressure values similar to placebo controls. There were no clinically relevant differences between treatment groups across time in hematology, lipid, or metabolic panel endpoints. (Appendix 14.)

B. Animal Studies Support the Safety of DMAA

DMAA toxicology has been studied in mice by intraperitoneal and intravenous administration.[38] These data were compared with data in mice for other naturally occurring and commonly consumed compounds (caffeine and phenethylamine). The results of this comparison

[38] Marsh DF, Howard A, Herring DA, *The Comparative Pharmacology of the Isomeric Nitrogen Methyl Substituted Heptylamines*, Journal of Pharmacology and Experimental Therapeutics, volume 103, at 325-329 (November 1951) (Appendix 15).

showed that DMAA has similar toxicity to caffeine (a commonly consumed and safe dietary ingredient) as well as phenethylamine (a constituent of chocolate).[39] In a recently completed toxicology study, the oral LD-50 of DMAA was determined to be approximately 481 mg/kg in rats and 324 mg/kg in rabbits -- showing less than half the toxicity of caffeine, which has an oral LD-50 of approximately 192 mg/kg in rats and 224 mg/kg in rabbits. An oral subchronic 90-day study in rats is scheduled to be completed within the next 60-90 days. Formal reports of both studies will be sent to you as soon as they are available.

C. FDA Has Previously Reviewed the Safety of DMAA as an Active Ingredient in a Drug Under an NDA and Authorized the Marketing of the Product for 30 Years Until It Was Voluntarily Withdrawn by the NDA Holder

On March 22, 1948, Eli Lilly submitted a new drug application (NDA) to FDA for a nonprescription drug, Forthane, with DMAA as the active ingredient. At that time, drug companies were required to submit an NDA demonstrating only that a drug was safe. On April 12, 1948, 39 days before the end of the 60-day statutory period for FDA review, FDA affirmatively authorized Lilly to begin marketing Forthane in the United States as a safe nasal decongestant.[40] Following FDA authorization, Lilly marketed Forthane from 1948 until it

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[39] Senda S, Hirota K, *Pyrimidine Derivatives and Related Compound. XXII, Synthesis and Pharmacological Properties of 7-deazaxanthine Derivatives*, Chemical and Pharmaceutical Bulletin (Tokyo), volume 22, at 1459-1467 (July 1974) (Appendix 16); Jackson DM, *The Interaction Between Beta-phenethylamine and Agents which Affect the Cholinergic Nervous System on Locomotor Activity and Toxicity in Mice*, Arzneimittelforschung, volume 24, no. 1, at 24-27 (January 1974) (Appendix 17).

[40] FD&C Act Section 201(ff)(3)(B) excludes from the definition of a dietary supplement a product that contains an article that is approved or authorized for investigation as a new drug. Section 201(ff)(3)(B) does not apply to DMAA because DMAA is not approved or authorized for investigation as a new drug.

voluntarily discontinued the product in 1978. DMAA was thus on the market for 30 years. There is no published literature suggesting that Forthane was unsafe. Even after Lilly withdrew Forthane from the market for its own commercial reasons, FDA did not take the technical step of withdrawing approval of the NDA until five years later.

D. An Independent Evaluation of DMAA by Environ Corporation Supports the Safety of DMAA

Environ International Corporation, a leading scientific consulting firm, reviewed all of the available data relevant to the assessment of human health and safety regarding the use of DMAA as a dietary ingredient. The company prepared a safety evaluation[41] authored by, among others, Joseph V. Rodricks, Ph.D., DABT, who is a founder of Environ and an internationally recognized expert in toxicology and risk analysis. Before founding Environ, Dr. Rodricks served as a scientist for FDA for 15 years, where he began as a toxicologist reviewing the safety of food ingredients. During his last four years at FDA, Dr. Rodricks served as Associate Commissioner for Health Affairs. Dr. Rodricks is a member of the Institute of Medicine of the National Academy of Sciences.

The Environ assessment concludes that "there is no scientific evidence that the labeled use of [DMAA products] by healthy adults will compromise individual health or increase susceptibility to heat-related injuries." In developing the safety assessment, Environ reviewed the following data: a literature search performed by Environ of the PubMed database and ToxNex search engine for published studies of DMAA (and chemical nomenclature synonyms),

[41] Joseph V. Rodricks et al., *Safety Evaluation of 1,3-Dimethylamylamine (DMAA) in Dietary Supplement Products* (May 2012) (Appendix 18).

and our dietary supplements containing DMAA; six publications (seven studies total) of human clinical studies of DMAA in healthy men and women; four studies in animals and humans published from 1927 to 1953; the U.S. patent for aminoalkanes; a safety assessment of DMAA performed by Cantox Health Sciences International; and FDA adverse event reports on dietary supplements. The Environ Report concludes that:

> hemodynamic effects (heart rate and blood pressure), or lack thereof, were similar across the studies regardless of whether subjects ingested DMAA, caffeine, DMAA and caffeine, [or our DMAA-containing dietary supplements].

Specifically, the Environ Report concluded that the blood pressure changes associated with DMAA and caffeine "are offset by reduced heart rate (4-5 bpm) to maintain consistent cardiovascular load."

Environ was unable to find any scientific or medical evidence that DMAA is unsafe when used according to the labeled Directions for Use and Warnings. Environ's exhaustive review of all of these materials and its conclusion that DMAA is a safe dietary ingredient provides strong support for determining that DMAA does not present a significant or unreasonable risk of illness or injury when taken as directed.

E. An Independent Evaluation of DMAA by Cantox Health Sciences Corporation Supports the Safety of DMAA

Cantox, another leading scientific consulting firm, also undertook an independent safety review of DMAA.[42] Cantox's conclusions are the same as those detailed in the Environ

[42] Intertek Health Sciences International, *supra* n. 5.

assessment: Used as directed, "it is unlikely that DMAA consumption from these products would cause adverse effects when used . . . by the appropriate population." The Cantox Report evaluated the publicly available data on DMAA, which included metabolic data, pharmacological data, animal data, and human clinical studies.

F. The Stimulant Effect of DMAA When Used as Directed is Comparable to the Stimulant Effect of 2 to 3 Cups of Coffee

The safety of DMAA as a dietary ingredient is further supported by the fact that the stimulatory hemodynamic effects, including short term increases in blood pressure, when used as directed, are statistically identical to those from the amount of caffeine in 2 to 3 cups of coffee.[43] Environ has documented the comparable effects of caffeine and DMAA in the attached analysis.[44] As Environ concludes, "caffeine and DMAA have both exhibited very good tolerance by adults," and the clinical trials using the combination of both substances "do not indicate that consumption of both compounds...would increase the susceptibility of adults to adverse cardiovascular events while exercising."

G. The Adverse Event Reports Received by FDA and the Company for DMAA Do Not Reveal a Single Serious Adverse Event Resulting from use in Accordance with the Labeled Directions for Use and Warnings

We have a robust system in place to receive and analyze consumer complaints and adverse event reports alleged to be associated with our products. Analyses of all such data and information we received as of the date of the Roosevelt letter reveals not one serious adverse

[43] Joseph V. Rodricks, *supra* note 33, at 9.

[44] Environ International Corporation, *A Comparison of the Physiological Effects of Caffeine and Dimethylamylamine (DMAA)* (May 8, 2012) (Appendix 19).

event report associated with the use of the company's products under the conditions of use recommended or suggested in the labeling.

1. Mandatory Adverse Event Reports Made by Us

As a preliminary matter, we emphasize that both the governing law[45] and FDA guidance[46] make clear that the submission of a serious adverse event report to the agency is not an admission that the dietary supplement involved caused or contributed to the adverse event being reported. The manufacturer, packer, or distributor whose name appears on the label of a dietary supplement marketed in the United States is required to submit to FDA all reports it receives alleging that a serious adverse event was associated with the use of a dietary supplement product, whether or not the alleged events are in fact causally related to its product.[47] Thus, in accordance with the law, upon receiving three reports alleging serious adverse events, we forwarded those reports to FDA. We do not believe, however, that any of the three incidents are evidence of a genuine safety issue associated with the product.

First, in all three cases, the consumers failed to use the product as labeled in the Directions for Use, failed to follow the label Warnings, or both. For example, a mandatory report submitted in March 2010 involved the use by a 16-year-old of a product with a black box warning prominently displayed on the principal display panel and other warnings repeated in dosing instructions stating that the product is not for use by individuals 18 years or younger.

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[45] FD&C Act § 761(g).

[46] FDA/CFSAN, *Guidance for Industry: Questions and Answers Regarding Adverse Event Reporting and Recordkeeping for Dietary Supplements as Required by the Dietary Supplement and Nonprescription Drug Consumer Protection Act* (Revised June 2009).

[47] FD&C Act § 761(b).

Similarly, the other two reports involved consumption of caffeine at the same time as use of the product, in spite of the labeled warnings that clearly state that it should not be used with any other stimulants or combined with other products containing caffeine.

More importantly, it is unclear that the ingestion of the product played a role in any of the three incidents. Indeed, in one of the three incidents, the person reporting the incident "believed" the subject had been taking the product for a period of time prior to the incident, but could not confirm whether the subject had consumed the product prior to the incident and did not state that the product played a role in the events that transpired. In another incident, the subject was performing exercise in extreme heat. In another, the consumer was taking a powerful prescription medicine that has been associated with sudden cardiac death and cardiac arrhythmia. The subject was also obese, based on his reported body weight and height. These reports do not provide data or information to suggest that DMAA was responsible for the incidents.

More fundamentally, and as discussed more fully above, the safety of DMAA has been well documented in seven clinical studies, none of which involved any serious adverse events and none of which demonstrated any statistically significant effect of the product upon cardiovascular risk factors such as those alleged to be linked to the product in two of the serious adverse event reports. As such, we do not believe that any of the mandatory adverse event reports submitted to FDA constitute evidence of a genuine safety issue caused by the product.

Finally, it has been brought to our attention that a serious adverse event report was made to FDA by another dietary supplement company, Iovate, regarding an incident that involved the ingestion of the same product in addition to numerous other supplements. However, the incident did not meet the statutory definition of a "serious adverse event," in

Section 761(a)(2) of the FD&C Act. A serious adverse event is defined as one that results in death, a life-threatening experience, inpatient hospitalization, a persistent or significant disability or incapacity, or a congenital anomaly or birth defect.[48] The subject of the Iovate report claimed to have experienced chest pain and heart fluttering, but did not seek a medical evaluation and was not admitted to the hospital. The incident therefore did not constitute a serious adverse event as defined by the statute. In addition, the consumer took the product in clear violation of the labeled Directions for Use and Warnings. He consumed it while taking other supplements and while consuming caffeine. In light of these facts, the report submitted by Iovate does not suggest that DMAA poses a safety issue when used in accordance with its labeled conditions of use.

2. Voluntary Adverse Event Reports Made By Other Parties

As of the date we received the Roosevelt letter, we were aware of 21 voluntary adverse event reports filed with FDA regarding events allegedly associated with the use of DMAA. These 21 reports were made between the years 2009 and 2012, during which time an estimated one billion servings of dietary supplements containing DMAA were sold by the dietary supplement industry in the United States. These reports do not support a causal relationship of DMAA with serious health risks for a number of reasons.

First, 12 of the 21 reports involved use of the product in violation of its labeled Directions for Use and Warnings, including dosages greater or more frequent than those in the

[48] FD&C Act § 761(a)(2). In addition, a serious adverse event could be one that required, based on reasonable medical judgment, a medical or surgical intervention to prevent the aforementioned serious outcomes.

labeled dosage instructions and uses in conjunction with other supplements, stimulants, and medications.

Second, at least nine of these incidents did not meet the statutory definition of a "serious adverse event." As noted above, a "serious adverse event" is defined in Section 761(a)(2) of the FD&C Act as one that results in death, a life-threatening experience, inpatient hospitalization, a persistent or significant disability or incapacity, or a congenital anomaly or birth defect.[49] A number of the voluntary reports alleged reactions falling far below that standard, including allegations of acne breakouts, tongue numbness, and pupil dilation.

Finally, the information included in these voluntary reports do not establish a causal relationship with the use of our products. A number of the voluntary reports variously included no information as to duration of use or dosage of our product, age of the consumer involved, or any relevant diagnostic values. More significantly, as discussed above, clinical trials demonstrate no adverse events or statistically significant alterations in cardiac function or blood pressure from the administration of DMAA. As such, the voluntary adverse event reports demonstrate no evidence of genuine safety concerns associated with our DMAA products.

V. Conclusion

For the reasons set forth in this letter, DMAA is a lawful dietary ingredient that is used in compliance with the requirements of the FD&C Act, as amended by DSHEA.

[49] FD&C Act § 761(a)(2).

VI. Confidentiality

The information in this submission constitutes trade secrets and confidential information that is exempt from public disclosure under 5 U.S.C. § 522. FDA is prohibited from disclosing this information pursuant to 18 U.S.C. § 1905 and Section 301(j) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 331(j).

Sincerely yours,

Jonathan V. Doyle
President

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– ORIGIN C19: from **BORAX**[1], on the pattern of *carbon* (which it resembles).

boronia /bə'rəʊnɪə/ ● n. a sweet-scented Australian shrub, cultivated for its perfume and flowers. [Genus *Boronia*.]

– ORIGIN mod. L., named after the Ital. botanist Francesco *Borone*.

borosilicate glass /ˌbɔːrəʊ'sɪlɪkeɪt/ ● n. a low-melting glass made from a mixture of silica and boric oxide.

borough /'bʌrə/ ● n. **1** Brit. a town (as distinct from a city) with a corporation and privileges granted by a royal charter. ➤historical a town sending representatives to Parliament. ➤an administrative division of London. **2** a municipal corporation in certain US states. ➤each of five divisions of New York City.

– ORIGIN OE *burg*, *burh* 'fortress, citadel', later 'fortified town', of Gmc origin, cf. **BURGH**.

borrow ● v. **1** take and use (something belonging to someone else) with the intention of returning it. ➤take and use (money) from a person or bank under agreement to pay it back later. **2** Golf allow (a certain distance) when playing a shot to compensate for a slope or other irregularity. ● n. Golf a slope or other irregularity on a golf course.

– PHRASES **be (living) on borrowed time** be surviving against expectations. **borrow trouble** N. Amer. take needless action that may have detrimental effects.

– DERIVATIVES **borrower** n. **borrowing** n.

– ORIGIN OE *borgian*, of Gmc origin.

borrow pit ● n. a pit resulting from the excavation of material for use in embankments.

Borsalino /ˌbɔːsə'liːnəʊ/ ● n. (pl. **-os**) trademark a man's wide-brimmed felt hat.

– ORIGIN C20: from the name of the manufacturer.

borscht /bɔːʃt/ (also **borsch** /bɔːʃ/) ● n. a Russian or Polish soup made with beetroot.

– ORIGIN from Russ. *borshch*.

borstal /'bɔːst(ə)l/ ● n. Brit. historical a custodial institution for young offenders.

– ORIGIN C20: named after the village of *Borstal* in southern England, where the first of these was established.

bort /bɔːt/ (also **boart**) ● n. inferior diamonds used in cutting tools.

– ORIGIN C17: from Du. *boort*.

borzoi /'bɔːzɔɪ/ ● n. (pl. **borzois**) a large Russian wolfhound of a breed with a narrow head and silky coat.

– ORIGIN C19: from Russ. *borzoĭ* (adj.), *borzaya* (n.), from *borzyĭ* 'swift'.

boscage /'bɒskɪdʒ/ (also **boskage**) ● n. a mass of trees or shrubs.

– ORIGIN ME: from OFr., ult. of Gmc origin.

bosh ● n. informal nonsense.

– ORIGIN C19: from Turk. *boş* 'empty, worthless'.

bosky /'bɒskɪ/ ● adj. poetic/literary covered by trees or bushes.

– ORIGIN C16: from ME *bosk*, var. of **BUSH**[1].

Bosnian /'bɒznɪən/ ● n. a native or inhabitant of Bosnia. ● adj. of or relating to Bosnia or its people.

bosom ● n. **1** a woman's breast or chest. **2** the breast as the seat of emotions: *quivering dread was settling in her bosom*. ➤a person's loving care or protection: *Bruno went home each night to the bosom of his family*. ● adj. (of a friend) very close.

– DERIVATIVES **-bosomed** adj. **bosomy** adj.

– ORIGIN OE *bōsm*, of W. Gmc origin.

boson /'bəʊzɒn/ ● n. Physics a subatomic particle, such as a photon, which has zero or integral spin.

– ORIGIN 1940s: named after the Ind. physicist S. N. *Bose* + **-ON**.

boss[1] informal ● n. a person who is in charge of an employee or organization. ● v. give orders in a domineering manner. ● adj. N. Amer. excellent.

– ORIGIN C19 (orig. US): from Du. *baas* 'master'.

boss[2] ● n. **1** a stud on the centre of a shield. **2** Architecture an ornamental carving at the point where the ribs in a ceiling cross. **3** the central part of a propeller. **4** Geology a large mass of igneous rock protruding through other strata.

– ORIGIN ME: from OFr. *boce*.

boss[3] ● n. US informal a cow.

– ORIGIN C19: of unknown origin.

bossa nova /ˌbɒsə 'nəʊvə/ ● n. a dance like the samba, originating in Brazil. ➤a piece of music for this dance.

– ORIGIN 1960s: from Port., from *bossa* 'tendency' and *nova* (fem. of *novo*) 'new'.

boss-eyed ● adj. Brit. informal cross-eyed; squinting.

– ORIGIN C19: cf. dial. *boss* 'miss, bungle'.

bossism ● n. US the controlling of a political party by party managers.

boss-shot ● n. dialect or informal a bad shot or aim.

– ORIGIN C19: from dial. *boss* 'miss, bungle'.

bossy[1] ● adj. **(-ier, -iest)** informal fond of giving orders; domineering.

– DERIVATIVES **bossily** adv. **bossiness** n.

bossy[2] ● n. (pl. **-ies**) N. Amer. informal a cow or calf.

– ORIGIN C19: of unknown origin.

bossyboots ● n. Brit. informal a bossy person.

Boston ● n. **1** a card game resembling solo whist. **2** a variation of the waltz or the two-step.

Boston crab ● n. Wrestling a hold in which a wrestler sits astride a prone opponent and pulls upwards on the opponent's legs.

Bostonian ● n. a native or inhabitant of Boston, the state capital of Massachusetts. ● adj. of or relating to Boston.

Boston ivy ● n. a Virginia creeper with three-lobed leaves, cultivated for its foliage. [*Parthenocissus tricuspidata*.]

Boston terrier ● n. a small terrier of a breed originating in Massachusetts from a crossing of the bulldog and terrier.

bosun /'bəʊs(ə)n/ (also **bo'sun**) ● n. variant spelling of **BOATSWAIN**.

bot[1] ● n. the larva of a botfly.

– ORIGIN C16: prob. of Low Ger. origin.

bot[2] ● n. Computing an autonomous program on a network which can interact with systems or users, especially in the manner of a player in some computer games.

– ORIGIN 1980s: shortening of **ROBOT**.

bot. ● abbrev. **1** botanic; botanical; botany. **2** bottle. **3** bought.

botanical ● adj. of or relating to botany. ● n. a substance obtained from a plant and used as an additive.

– DERIVATIVES **botanically** adv.

botanic garden (also **botanical garden**) ● n. a place where plants are grown for scientific study and display to the public.

botanizing (also **-ising**) ● n. the studying of plants in their natural habitat.

– DERIVATIVES **botanize** v.

Botany /'bɒt(ə)nɪ/ (also **Botany wool**) ● n. merino wool.

– ORIGIN C19: named after *Botany* Bay in Australia, from where the wool orig. came.

botany /'bɒt(ə)nɪ/ ● n. the scientific study of the structure, ecology, distribution, classification, and economic importance of plants. ➤the plant life of a particular region or geological period.

– DERIVATIVES **botanic** adj. **botanist** n.

– ORIGIN C17: from earlier *botanic* (from Fr. *botanique*) based on Gk *botanikos*, from *botanē* 'plant') + **-Y**[3].

botch informal ● v. carry out (a task) badly or carelessly. ● n. (also **botch-up**) a badly carried out task.

– DERIVATIVES **botcher** n.

– ORIGIN ME (in the sense 'repair' but orig. not implying clumsiness): of unknown origin.

botel ● n. variant spelling of **BOATEL**.

botfly /'bɒtflaɪ/ ● n. (pl. **-flies**) a stout hairy-bodied fly whose larvae are internal parasites of horses and other mammals. [*Gasterophilus* and other genera.]

both ● predet., det., & pron. two people or things, regarded and identified together. ● adv. applying equally to each of two alternatives.

– ORIGIN ME: from ON *báthir*.

bother ● v. **1** [with neg.] take the trouble to do something. **2**

worry, disturb, or upset (sor
concern about or interest in.
of trouble or fuss. ● exclam. chi
irritation.
ORIGIN C17 (as a noun in the
of Anglo-Ir. origin; prob. re
bodhraim 'deafen, annoy'.

botheration informal, dated ● r
express irritation.

bothersome ● adj. annoying

both ways ● adv. & adj. anotl
PHRASES **have it both ways**
ible ways of thinking or beha

bothy /'bɒθi/ (also **bothie**) ●
small hut for farm labourers
ORIGIN C18: obscurely rel. t
bothan, and perh. to **BOOTH**.

boto ● n. variant spelling of **B**

Botox /'bəʊtɒks/ ● n. tradema
botulin, used medically to tr
ditions and cosmetically to re
arily paralysing facial muscle
ORIGIN from **BOTULINUM TOXI**

bo tree /bəʊ/ (also **bodh tre**
India and SE Asia, regarded
[*Ficus religiosa*.]
ORIGIN C19: representing Sinh
ledge' (Buddha's enlightenme
such a tree), from *bō* (from
thoroughly') + *gaha* 'tree'.

botryoidal /ˌbɒtrɪ'ɔɪd(ə)l/ ●
shaped like a cluster of grape
ORIGIN C18: from Gk *botruoei*
grapes') + **-AL**.

botrytis /bə'traɪtɪs/ ● n. a gre
erately cultivated on the grap
ORIGIN mod. L. (name of a g
botrus 'cluster of grapes').

Botswanan /bɒ'tswɑːnən/ ● n
Botswana, a country of south
lating to Botswana.

botte /bɒt/ ● n. Fencing an attac
ORIGIN from Fr.

bottle ● n. **1** a container with
storing liquids. ➤a large metal
gas. ➤a baby's feeding bottle.
or confidence. ● v. **1** place in b
something up) repress or c
out) Brit. informal lose one's ne
something. **4** informal hit with a
PHRASES **bottle and glass** Br
bottle informal start to drink al
ORIGIN ME: from OFr. *boteill*
dimin. of late L. *buttis* (see **BU**

bottle bank ● n. Brit. a place
may be deposited for recycling

bottle blonde ● adj. (of a wo
blonde that looks dyed. ● n. a

bottlebrush ● n. **1** a cylindri
side bottles. **2** an Australian
spikes of scarlet or yellow f
brushes. [Genus *Callistemon*.]

bottle-feed ● v. feed (a baby)

bottle green ● n. dark green.

bottle jack ● n. NZ a large jac

bottleneck ● n. **1** the neck
narrow section of road where
a device worn on a guitarist's
sliding effects on the strings.

bottlenose dolphin (also **bo**
a stout-bodied dolphin with a
in tropical and temperate
truncatus.]

bottlenose whale (also **bo**
beaked whale with a bul
Hyperoodon: two species.]

bottler ● n. **1** a person who bo

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Alan Davidson

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Caucasus, but incorporated in dishes rather than served as a sauce.

Walnuts are liberally used. Georgia is almost the only country in the world where this rich, oily nut is used as an integral part of daily cooking and not just as a garnish.

Meat is often cooked in chunks on skewers, i.e. as the *shashlyk* or *shish kebab* (see KEBAB) which is ubiquitous in the region; or in the stew called *Chankhi* where a little meat is cooked with a lot of vegetables and often with rice too; or cooked by frying to go into the sort of stew called *chakhokhbili* (often made with chicken).

Among vegetables, there is a noticeable enthusiasm for beans, especially lubia (LABLAB BEANS). These are prepared with plenty of spices and herbs, e.g. the following collection: cardamom, parsley, celery, leek, mint, basil or dill, coriander, cinnamon, cloves or saffron, and black pepper. Liberal use of such flavourings is characteristic of Georgian cuisine generally.

The numerous rice dishes called *plov* are patently related to PILAF, and equally variegated. However, although rice is important, there is no doubt about what is the real staple for Georgians, and that is bread. It has been said that for all Caucasians a meal without bread is unthinkable. The oldest bread is the thin, crisp, cracker-like bread known as LAVASH baked in a TANDOOR (known as *toné* to Georgians). The numerous other breads include *peda* (like PITTA bread), and Georgian specialities such as *mchadi* (a round flat corn bread), *kachapuri* (bread stuffed with cheese before being baked), and *deda's puri* (meaning mother's bread).

Cheeses in great variety constitute another strength of the Georgian kitchen. *Brynza*, a brine cheese, is popular and may be fried and served with the corn bread mentioned above and herbs. However, most cheese is probably eaten in the form of ZAKUSKI.

geranium the common and familiar name of popular garden plants of the genus *Pelargonium* (there is a genus *Geranium*, to which some small-flowered plants like the cranesbill geranium belong, but most garden geraniums are *Pelargonium* spp).

Geraniums, which are native to southern or tropical Africa, were introduced to Europe in 1690. The leaves (not the flowers) of a number of species, especially *Pelargonium capitatum* and *P. odoratissimum*, have a roselike scent. This is because they contain the same essential oils, geraniol and citronellol, as attar of roses (see ROSES). The proportions of these vary with the strain of the plant, soil conditions, and degree of maturity; and other essential oils are often present. So the scent of geraniums may be lemony, or like orange, apple, or nutmeg, besides resembling rose. Since the mid-19th century the rose-scented geranium has been cultivated for the production of 'rose geranium oil', which is used in perfumes but also for flavouring and scenting food, in the manner of rosewater. In Tunisian confectionery geranium water largely replaces rosewater.

The addition of geranium leaves, in small quantity, to fruit desserts or to confections such as creams and sorbets is effective.

German cheeses viewed collectively, are a disappointment. Before the reunification of the country, W. Germany alone ranked fourth of the countries of the world in cheese production; but the originality and variety of German cheeses does not match this lofty position.

Many German cheeses have external inspiration, like the EMMENTAL which has been made with success ever since Swiss experts were summoned in the 1840s to give advice on it to the cheese-makers of the Allgäu region. The same applies to LIMBURGER, the best known of the strong-smelling cheeses for which Germans seem to have a special liking; it had its origin in Belgium (although ROMADUR, its odoriferous partner, came from Bavaria). And the German Münster is a version of the French MUNSTER. The origins of TILSITER are more confusing. It was first made at Tilsit, when that city was in E. Prussia, but by Dutchmen.

Other points of interest in the German cheese list include the innovative Camboloza (resembling but not to be confused with Bavarian blue); the range of HAND CHEESES; and, most popular of all, the multiple forms of QUARK.

One way in which German cheeses have been important is in their influence on American cheeses, which has been greater than one might expect; this is mainly due to the large numbers of farmers of German descent who have played a prominent part in the American dairy industry of the Mid-western States.

German cookery books have a distinguished history. From the late 15th to the 17th centuries they were more numerous and impressive than French ones, and rivalled those of Italy. The earliest and possibly the most famous is the *Küchenmeisterei*. This was first published in 1485 and, amazingly, had appeared in more than a dozen editions by 1500. No other printed cookery book of the 15th century had such an impressive record. And this book continued to sell well for more than a century afterwards. Uta Schumacher-Voelker (1980), in her admirable essay on printed German cookery books up to 1800, relates it to the political and economic context in which it was compiled, and explains why it seems almost certain that the compilation was first made in a S. German monastery, citing the importance placed on Lenten food and other relevant features:

> the complete lack of hare despite other game recipes (hare was regarded as unchaste); the lack of almost all saltwater fish (as monasteries mainly used their own products); the lack of sugar, which was very much in fashion at the time for the rich, and its substitution by honey (for whose production monasteries were famous); and the use of home-grown herbs rather than an abundance of expensive spices.

About a century later came Max Rumpoldt's *Ein neues Kochbuch* (1581), a finely produced and extensive work which contained something like 2,000 recipes, intended mainly for the upper class. Only one other cookery book of the century (Frantz de Rontzier's *Kochbuch*, 1598) displayed a comparable wealth of ingredients.

In the 17th century there was a period, corresponding to the Thirty Years War, when book production almost ceased. However, even before the end of the war some translations of foreign cookery books began to appear, including several from France and works by Sir Kenelm DIGBY and Hannah WOLLEY from England. The especially important work by Massialot appeared in German translation in 1739, and Menon followed in 1766. By this time many foreign culinary terms were being used in German works. Also, the first books with a regional emphasis had begun to appear. Maria Schellhammer (1692) was from N. Germany, while Conrad Hagger's book of Saltzburg cookery embodied recipes from what is now Austria. And there were many others in the 18th century; Schumacher-Voelker lists a selection of fifteen, in which Hamburg, Berlin, Nuremberg, Augsburg, Leipzig, and Magdeburg were among the cities represented.

Regional cookery books have continued to be important up to the present time. During the 19th and 20th centuries the appearance of cookery books in new genres matched what was going on in other European countries rather than, as in this instance, anticipating. A tribute must, however, be paid to the pioneering aspects of the gastronomic work by RUMOHR (1822).

READING: Adamson (2000, 2002).

German regional cookery is, as the succeeding entry makes clear, the essence of the national kitchen. Germany unified late and its food remains intensely regional: there is no national food. Certain elements, however, create the Teutonic nuance: rye bread, sour and skimmed milk cheeses, sausages and ham from the ubiquitous pig. Certain prejudices are as good as myths: you will see more SAUERKRAUT in France.

Germans observe the seasons. They consume asparagus from 1 May to 24 June;

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A STUDY ON THE CHEMICAL CONSTITUENTS OF GERANIUM OIL

Zang Ping Qing Jun Lu Qing
(Physical and Chemical Analysis Research Center)

Abstract In this paper, the chemical constituents of geranium oil, collected from Rong Jiang, Guizhou, are studied by means of capillary gas chromatography-mass spectrometry. Over 40 constituents are separated, of which 31 components consisting 95.07% of the oil, have been identified. The main ingredients are citronellol (24.73%), garaniol (8.79%), â-guaiene (8.97%), p-menthone (7.18%), and linalool (4.16%).

Keywords geranium oil, capillary gas chromatography-mass spectrometry

Introduction

Pelargonium graveolens is a perennial herb within the Geraniaceue family. It is produced in regions such as France, Egypt, La Reunion, Morrocco, the former USSR, and Japan. It is also grown all over China, with the volume grown in Yunan and Sichuan province being the largest. In Guizhou it is grown in Guiyang, Wangmo, and Rongjiang[1]. Germanium oil is obtained from fresh geraniums, pelaronium graveolens, rose germaniums and their subspecies. The harvesting period is determined by climate conditions in the production location. The processing period is usually from the middle of July to October. The essential oil yield is around 0.1-0.3%. Geranium oil has a rose and germanium aroma and a peppermint tinge. Its color ranges from light yellow to dark yellow. It is mainly used for adding scent to high-grade cosmetics, and for other rose essences. When used this way, it is rather economical[2].

In order to provide a scientific basis for breed selection and essential oil quality evaluation, we used capillary gas chromatography-mass spectrometry (GC-MS) to perform a qualitative and quantitative analysis of of the main components and determine the physical and chemical parameters of the geranium oil obtained from the Rongjiang Region of Guizhou Province. This will have certain significance for the development and utilization of Guizhou's fragrant plant resources, and will help increase the depth of understanding of essential oils.

1. Summary of Principles

Perfume essential oil components are extremely complex. They are compounds formed from organic compounds such as terpene, sesquiterpenes, aromatics, alicylics, and aliphatics. Previously, investigation into scents and essential oils was performed with standard physical and chemical methods. Not only was this slow but it was also difficult to accurately determine the compositions using these methods.

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Gas chromatography (GC) uses the matching of volatile substances in high molecular weight liquid and carrier gas to begin chemical separation, and then the spectrum can be determined with a detector. Since perfumes are formed from highly-volatile chemical compounds, gas chromatography is the most suitable method. Furthermore, since each organic compound has its own mass spectrum, mass spectrometry has strong qualitative capacity[3]. This experiment uses a combined capillary gas chromatography-mass spectrometry method. Each component in the mass spectrum will be separately input in order into a spectrograph to undergo mass spectrography analysis. At the same time, this will achieve the quantitative and qualitative objects of this experiment. Furthermore, complex quantitative operations such as spectrum temperature control, collection of spectrum data, data storage and mass spectrum image examination will be performed by a computer. Micro-technology has thus been applied to the process of perfume component analysis, from compound separation to chemical structure determination in a way that is fast and accurate.

2. Samples

Air-dry fresh pelargonium graveolens stem and leaves. Cut, and use the steam distillation method to retrieve the dark-yellow volatile oil, the oil yield is 0.15%, and the denstity is d^{20}_4= 0.895, and the index of refraction is n^{20}_D=1.465-1.473, optical rotation is a^{20}_D= 7°30' ~ –10°16'.

3. Equipment

The HP 5890 Gas Chromatography System Series I HP5989A MS Engine.

The main technological specifications are: mass range 10-1000amu (can be extended to 2000amu); sensitivity, EI hexachlorobenzene s/N >20, CI (chemical source) 100pg = Benzophenone s/N > 10; scan speed: maximum of 2000amu/s, resolution > 2500; ionization energy 10-250eV adjustable; mass stability 0.1amu/8hr.

4. Experimental Conditions

4.1 Gas Spectrometry Conditions

Crosslink 5% benzyl polysiloxane fused silica capillary column 30m x 0.25m; carrier gas: He 50mL/min; column pressure 12.0kPa; temperature of boil room: 240°C; Column temperature starts at 50°C, and raises 25°C/min up to 125°C, than a 10°/min increase to 250°C; stop for 7 minutes; Sample volume: 1ìL (Acetaldehyde solution dilute); diversion ratio 50:1.

4.2 Mass Spectra Conditions

Ion source temperature 250°C; separation method EI; electric potential 70eV; resolution 2500; connection temperature 280°C; scan speed 0.9s/full process; sweep mass range 40-500amu.

5. Results and Discussion

Using the GC-MS method more than 40 components were separated from the geranium oil. After MS analysis 31 chemical components have been determined as shown in the Figure. Using the surface chemical method to calculate their relative percentage content, the results are shown in Table 1.

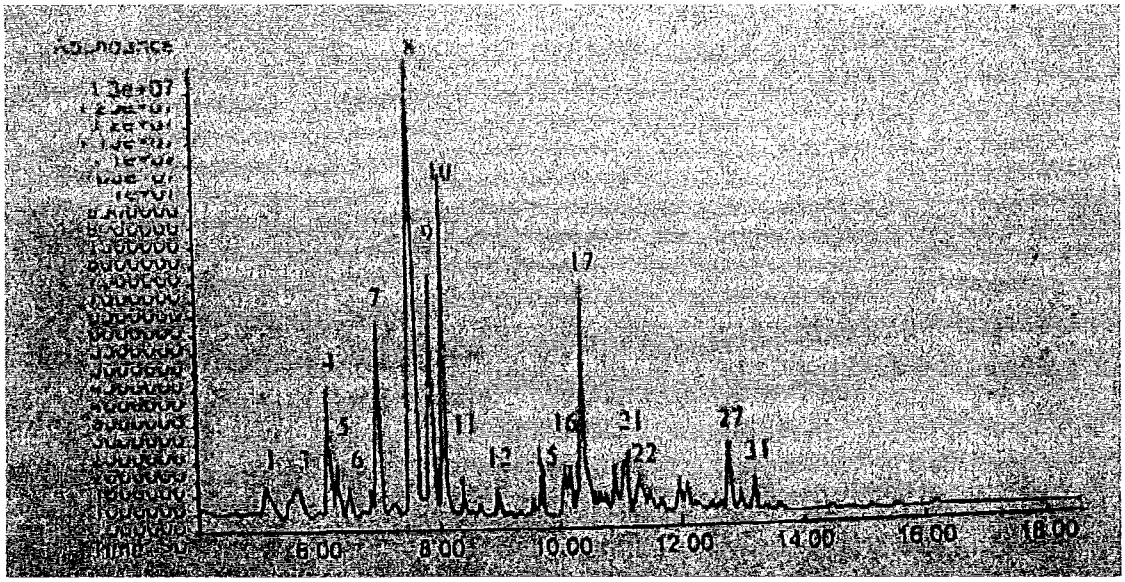

Figure – Geranium oil GC-MS Spectrum

Table 1 – Geranium oil chemical composition

Number/English compound/Chinese name/Molecular formula/Molecular weight/relative content (%)

	β-Myrcene	β-香叶烯	$C_{10}H_{16}$	136	1.96
	δ-4-Carene	δ-4-蒈烯	$C_{10}H_{16}$	136	0.64
	p-mentol	薄荷醇	$C_{10}H_{20}O$	154	4.16
	Rose' oxide	玫瑰醚	$C_{10}H_{18}O$	154	2.86
	2-Propenal,3-(dimethyl amino)-3-ethon'	3-(2-甲胺基)-3-乙氧基-2-丙醛	$C_5H_{11}O_2N$	143	0.82
6	α-3-Carene	α-3-蒈烯	$C_{10}H_{16}$	136	1.58
7	P-Menthone	p-薄荷酮	$C_{10}H_{18}O$	154	7.19
8	β-Citronellol	β-香茅醇	$C_{10}H_{20}O$	156	24.73
9	Geraniol	香叶醇	$C_{10}H_{18}O$	154	8.19
10	Citronellyl acetate	香茅醇乙酸酯	$C_{12}H_{22}O_2$	198	9.97
11	1-β-Pinene	1-β-蒎烯	$C_{10}H_{16}$	136	1.08
12	Citronellyl Propionate	香茅醇丙酸酯	$C_{13}H_{24}O_2$	212	2.31
13	α-methyl-Benzeneeth-anarmine	α-甲基-苯乙胺	$C_9H_{13}N$	135	0.19
14	α-Ylangene	α-依兰烯	$C_{15}H_{24}$	204	0.57
15	Calarene	白菖蒲烯	$C_{15}H_{24}$	204	1.65
16	Caryophyllene	石竹烯	$C_{15}H_{24}$	204	1.62
17	β-Guajene	β-愈创木烯	$C_{15}H_{24}$	204	8.97
18	α-Humulene	α-葎草烯	$C_{15}H_{24}$	204	0.68
19	N-methyl-Benzen-eethanarmine	N-甲基-苯乙胺	$C_9H_{13}N$	135	1.59
20	α-Cubebene	α-荜澄茄烯	$C_{15}H_{24}$	204	0.79
21	γ-Cadinen	γ-杜松烯	$C_{15}H_{24}$	204	1.76
22	α-Elemene	α-榄香烯	$C_{15}H_{24}$	204	1.75
23	δ-Cadinen	δ-杜松烯	$C_{15}H_{24}$	204	2.17
24	Geranyl acetate	香叶醇乙酸酯	$C_{12}H_{20}O_2$	196	1.13
25	Propanoic acid, phenylethyl ester	丙酸-2-乙酸苯乙酯	$C_{11}H_{14}O_2$	192	1.41
26	N-methyl-2-hydroxyty-ramine	N-甲基-2-羟基酪胺	$C_9H_{13}NO$	151	1.02
27	Cyclohexene, 1-methyl-4-(1-methylethe)	1-甲基-4-异丙基-环己烯	$C_{10}H_{18}$	138	2.07
28	Tricyclene	三环萜	$C_{10}H_{16}$	136	1.03
29	5-Pyrimidinamine, 2-methyl-	5-甲基-2-胺嘧	$C_5H_6N_2$	129	0.23
30	4-Hexenamide, N-methyl-	4-甲基-2-己酰	$C_7H_{13}N$	115	0.60
31	5-Hexenamide, 5-methyl-	5-甲基-2-己酰	$C_7H_{13}N$	129	0.29

Total surface content which is currently determined – 95.07

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香叶油化学成份的研究

章平 秦军 吕晴

(贵州工学院理化分析研究中心)

摘 要 采用毛细管气相色谱—质谱—计算机联用法研究了贵州榕江地区香叶油的化学成分。色谱分离出40多个组分,质谱鉴定了31个成分,占该挥发油总量的95.07%。其主要成分是香茅醇(24.73%)、香叶醇(8.19%)、香茅醇乙酸酯(9.97%)、β-愈创木烯(8.97%)、P-薄荷酮(7.19%)和芳樟醇(4.16%)等。

关键词 香叶油;毛细管气相色谱—质谱

中图法分类号 O657.71; TQ 651.2

引 言

香叶天竺葵(*Pelargonium graveolens*)为生牻儿苗科(*Geraniaceue*)植物,多年生草本。原产法国、埃及、留尼旺岛、阿尔及利亚、摩洛哥,前苏联和日本等地。我国各地均有栽培,以云南、四川两省栽培面积最大。我省贵阳、望谟、榕江地区也有栽培[1]。香叶油(*Geranium Oil*)是从新鲜的、整株的香叶植物—香叶天竺葵和玫瑰天竺葵及其亚种中获得。香叶的收割期取决于生长地区的气候条件,加工期一般从每年七月中旬到十月。精油的得率为0.1%~0.3%。香叶油具有玫瑰和香叶的香气以及薄荷气息,颜色自淡黄至深黄色,主要用于高级化妆品加香及其它玫瑰型香精,具有较高的经济价值[2]。为了对香叶植物的品种选育和精油质量的评定工作提供科学依据,我们采用毛细管气相色谱—质谱—计算机联用法对贵州榕江地区的香叶油样进行了主要成分的定性定量分析及理化常数的测定,对贵州芳香植物资源的开发利用及精油的深加工有一定的意义。

1 原理简述

香料精油的成分十分复杂,它是由萜烯、倍半萜烯、芳香族、脂环族和脂肪族等多种有机化合物组成的混合物。过去对香料和精油的检测主要依靠一些常规的理化方法,不但速度慢而且很难弄清其中的组成。气相色谱法(GC)是利用挥发性物质在高分子液体(液相为固定相)和载气之间的分配进行分离、用检测器得到色谱的方法。因为香料是由挥发性强的化合物组成的,所以采用气相色谱法作为分析手段最为合适。又由于每一个有机化合物有它自己的质谱特性,所以质谱法(MS)具有强有力的定性能力[3]。

本实验采用气相色谱—质谱—计算机联用法,把色谱分离出来的每个成份按次序连续通进质谱仪中进行质谱分析,同时达到定性、定量的目的。且色谱程序升温控制,质谱离子碎片信

• 1995—09—25收稿。

息的采集，数据的贮存和质谱图的自动检索，定量计算等复杂的操作均由计算机按指令自动地进行工作。它使香料精油的成份分析从组分的分离到化学结构的鉴定真正实现了微量、快速、精确。

2 样　品

将新鲜的香叶天竺葵枝和叶略为风干，剪碎，用水蒸汽蒸馏法提取得到淡黄色挥发油，出油率为 0.15%，密度 $d_4^{20}=0.895$，折光率 $n_D^{20}=1.465\sim1.473$，旋光度 $\alpha_D^{20}=-7°30'\sim-10°16'$。

3 仪　器

美国惠普公司 HP5890 Ⅱ 型气相色谱—HP5989A 型质谱联用仪。其主要技术指标为：质量范围 10～1000 amu(可扩大到 2000 amu)；灵敏度：EI(电子轰击)50Pg 六氯苯 s/N＞20，Cl(化学源)100 pg＝苯甲酮 s/N＞10；扫描速度：最大到 2000 amu/s；分辨率＞2500；电离能：10～250 eV 可调，质量稳定性 0.1 amu/8hr。

4 实验条件

4.1 气相色谱条件

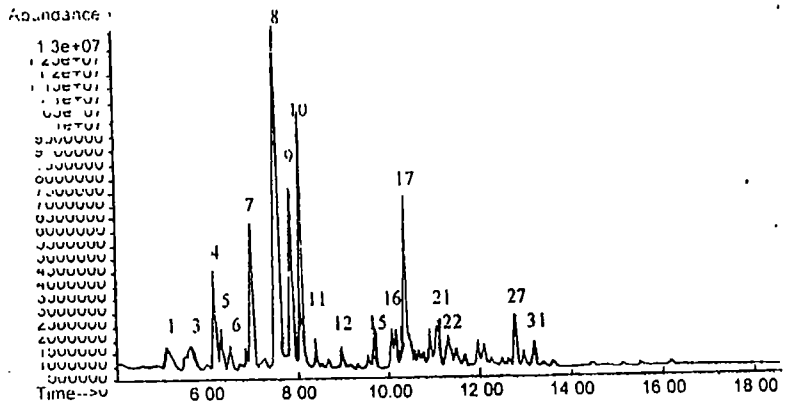
交联 5%苯甲基聚硅氧烷弹性石英毛细管柱，30 m×0.25 mm；载气：氦气 He，50 mL/min；柱前压 12.0 kPa；汽化室温度 240 ℃；柱温从初温 50 ℃以 25 ℃/min 升到 125 ℃，再以 10 ℃/min 升至 250 ℃，停留 7 分钟；进样量：1 μL(无水乙醇稀释)；分流比 50:1。

4.2 质谱条件

离子源温度 250℃；电离方式 EI；电子能量 70 eV；分辨率 2500；接口温度 280 ℃；扫描速度 0.9 s/全程；扫描质量范围 40～500 amu。

5 结果与讨论

用气相色谱—质谱法从香叶油中分离出 40 多个组分，经质谱分析确定了 31 个化学成份，如附图所示。用面积归一化法计算其相对百分含量，分析结果列于表 1。



附图　香叶油 GC/MS 总离子流色谱图

表 1 香叶油化学成份

峰号	化合物英文名称	中文名称	分子式	分子量	相对含量(%)
1	β-Myrcence	β-香叶烯	$C_{10}H_{16}$	136	1.96
2	δ-4-Carene	δ-4-蒈烯	$C_{10}H_{16}$	136	0.64
3	Linaluol	芳樟醇	$C_{10}H_{18O}$	154	4.16
4	Rose oxidi	玫瑰醚	$C_{10}H_{18O}$	154	2.86
5	2-Propenal,3-(dimethyl -mino)-3-ethon	3-(2-甲胺基)-3-乙氧基-2-丙醇	$C_7H_{13}O_2N$	143	0.82
6	σ-3-Carene	σ-3-蒈烯	$C_{10}H_{16}$	136	1.58
7	P-Menthone	p-薄荷酮	$C_{10}H_{18O}$	154	7.19
8	β-Citronellol	β-香茅醇	$C_{10}H_{20}O$	156	24.73
9	Geraniol	香叶醇	$C_{10}H_{18}O$	154	8.19
10	Citronellyl acetate	香茅醇乙酸酯	$C_{12}H_{22}O_2$	198	9.97
11	1-β-Pinene	1-β-蒎烯	$C_{10}H_{16}$	136	1.08
12	Citronellyl Propionate	香茅醇丙酸酯	$C_{13}H_{24}O_2$	212	2.31
13	α-methyl-Benzeneeth-anarmine	α-甲基-苯乙胺	$C_9H_{13}N$	135	0.19
14	α-Ylangene	α-依兰烯	$C_{15}H_{24}$	204	0.57
15	Calarene	白菖油萜	$C_{15}H_{24}$	204	1.65
16	Caryophyllene	石竹烯	$C_{15}H_{24}$	204	1.62
17	β-Guaiene	β-愈创木烯	$C_{15}H_{24}$	204	8.97
18	α-Humulene	α-葎草烯	$C_{15}H_{24}$	204	0.68
19	N-methyl-Benzen-eethanarmine	N-甲基-苯乙胺	$C_9H_{13}N$	135	1.59
20	α-Cubebene	α-荜澄茄烯	$C_{15}H_{24}$	204	0.79
21	γ-Cadinen	γ-杜松烯	$C_{15}H_{24}$	204	1.76
22	α-Elemene	α-榄香烯	$C_{15}H_{24}$	204	1.75
23	δ-Cadinen	δ-杜松烯	$C_{15}H_{24}$	204	2.17
24	Geranyl acetate	香叶醇乙酸酯	$C_{12}H_{20}O_2$	196	1.13
25	Propanoic acid, 2-pheny-lethyl ester	丙酸-2-乙酸苯乙酯	$C_{13}H_{16}O_2$	192	1.41
26	N-methyl-2-hydro-xyty-ramine	N-甲基-2-羟基酪胺	$C_9H_{13}NO$	151	1.02
27	Cyclohexame, 1-methyl-4(1-methylethe)	1-甲基-4-异丙基-环已烯	$C_{10}H_{18}$	138	2.07
28	Tricyclene	三环萜	$C_{10}H_{16}$	136	1.03
29	2-Heptanamine,5-methyl-	5-甲基-2-庚胺	$C_8H_{19}N$	129	0.23
30	2-Hexanamide,4-methyl-	4-甲基-2-已胺	$C_7H_{17}N$	115	0.66
31	2-Hexanamide,5-methyl-	5-甲基-2-已胺	$C_7H_{19}N$	129	0.29
	已定性的组分占色谱总流出峰面积的百分数				95.07

结果表明，其主要成分为香茅醇、香叶醇、香茅醇乙酸酯、β-愈创木烯、P-薄荷酮和芳樟醇，其它成分含量低。据资料报道[4,5]，香叶油的醇部分主要以香叶醇和香茅醇为主，分别为 10%～20%和 30%～40%，酯部分以香茅醇和香叶醇的甲酸酯为主。贵州榕江地区香叶油中香茅醇和香叶醇的含量比国外同类产品稍低一些，但所含 β-愈创木烯，P-薄荷酮、芳樟醇和玫瑰醚的含量都较高，具有浓郁的橙花、玫瑰花香气和薄荷气息。酯部分是以香茅醇乙酸酯、丙酸酯和香叶醇乙酸酯的形式存在，而不是以甲酸酯的形式存在。另外，还有一定量的香叶烯、蒎烯、白菖油萜、杜松烯、榄香烯和石竹烯等萜类芳香物质，在化学组成上具有它的特点。这些物质虽然含量较低，但对于精油香气的贡献是十分重要的，它们赋于精油一种圆浑的天然香气。所以该香叶油具有良好的开发价值。

作为天然香料植物，由于其部位、产地、气候、栽培及管理方法、收割时间和提取方法的不同，其成份和比例也发生变化。本文采用色质谱计算机联用技术测定香叶油的化学成份，为进一步开发利用贵州野生香料资源及天然香料品种改良，加工工艺的改进，质量监控和成分分析提供了科学依据，具有一定的实用价值和参考意义。

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A STUDY ON THE CHEMICAL CONSTITUENTS OF GERANIUM OIL

Zang ping　Qing Jun　Lu Qing

(Physical and Chemical Analysis Research Center)

Abstract　In this paper, the chemical constituents of geranium oil, collected from Rong Jiang, Guizhou, are studied by means of capillary gas chromatography-mass spectrometry. Over 40 constituents are separated, of which 31 components, constituting 95. 07% of the oil, have been identified. The main ingredients are citronellol(24. 73%), garaniol(8. 79%), -guaiene(8. 97%), p-menthone(7. 19%) and linalool(4. 16%), etc.

Key Words　geranium oil, capillary gas chromatography-mass spectrometry

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HEALTH SCIENCES INTERNATIONAL

2233 Argentia Road, Suite 308
Mississauga, ON, Canada L5N 2X7
Phone: 905-542-2900
Fax: 905-542-1011

DMAA: Review of Safety Data and Occurrence in the Geranium Plant and its Essential Oil

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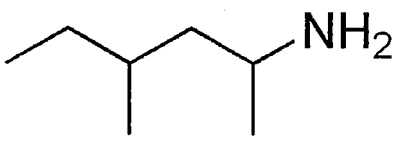
USPlabs, LLC
10761 King William Drive
Dallas, Texas
75220

At the request of USPlabs, LLC, Intertek Cantox has reviewed and assessed the safety data that is publicly available for 1,3-dimethylamylamine (DMAA, also known as 2-hexanamine-4-methyl), as well as data, both published and unpublished pertaining to the occurrence of DMAA in the geranium plant (*Pelargonium graveolens*) and its essential oil. Intertek Cantox was also requested to review recent work (confidential, unpublished report) indicating the DMAA is in fact present in samples of the tissues and essential oil of geranium plants.

DMAA is a component of two of USPlabs' dietary supplement formulations: (OxyELITE Pro™), a weight loss aid, and Jack3d™", a "pre-workout" supplement targeted to assist athletic training. The chemical structure of DMAA is shown in Figure 1.



The amount of DMAA in each serving of these products was likely in the range of [redacted] to [redacted] mg. It is our understanding that the suggested dosing rate is 2 to 3 servings per day. It is also our understanding that labels on each of OxyELITE Pro™ and Jack3d™ state the need for mandatory clearance from a physician, incorporation of an initial tolerance dosing period; maximum duration of use of 8 weeks; the requirement for the individual to be healthy, and avoid

using the product if they have various medical conditions, take certain medications, other dietary supplements, or if they wish to avoid caffeine. Intertek Cantox was requested to assess the safety of DMAA in general, and specifically within the context of its presence in the two formulations currently marketed by USPlabs.

The safety data on DMAA are reviewed in the order of data pertaining to DMAA alone, DMAA within USPlabs dietary supplement formulations OxyELITE Pro™ and Jack3d™, and DMAA in combination with caffeine. Following the discussion of the safety data, the occurrence of DMAA in the geranium plant (*Pelargonium graveolens*) is reviewed and assessed.

Safety of DMAA

Safety of DMAA Alone:

The publicly available safety data on DMAA was limited to general pharmacology and acute toxicity data in animals, and clinical trial and case report type data in humans. No metabolic data or long-term animal toxicity studies were found in the literature.

While no metabolic data were available for DMAA, its metabolism can be predicted from what is known about the metabolism of aliphatic amines in general. Once absorbed into the body, aliphatic amines in mammalian systems are metabolized by enzymes called flavin-containing monooxygenases. These enzymes insert an oxygen atom onto the carbon adjacent to the amine function to give an unstable compound called an imine. This imine is hydrolyzed to the aldehyde which is subsequently metabolized to a carboxylic acid and excreted in the urine (JECFA, 2006). The reaction sequence described above is referred to as oxidative deamination. While DMAA is likely to undergo some form of oxidative deamination, there are insufficient data to assess the bioavailability (*i.e.*, the amount of DMAA that actually enters systemic circulation following consumption) and whether or not DMAA retains pharmacologic activity following consumption.

Limited pharmacology data exist for DMAA. The relative potency of DMAA with respect to specific receptor activity is unknown. It has about 1/250th the potency of epinephrine with respect to pressor effects in pithed dogs (Swanson and Chen, 1946). DMAA was reported to have 35.2% less pressor activity relative to ephedrine, and 1/200th the activity of epinephrine, in pithed cats (Rohrmann and Shonle, 1944).

The acute toxicity of DMAA has been tested by the intraperitoneal route of exposure (*i.e.*, injection into the abdominal cavity) (Merck, 1989). The LD_{50} value was reported to be 185 mg/kg body weight in mice (Merck, 1989). This value has little relevance to oral consumption of DMAA. Normally, the LD_{50} value for the oral route of exposure would be greater (*i.e.*, less toxic) as compared to intraperitoneal injection. Oral LD_{50} values for aliphatic amines similar in structure to DMAA include: 545 mg/kg body weight for *sec*-butylamine and 200 to 500 mg/kg body weight for octanamine (Greim *et al.*, 1998). An oral LD_{50} value of 670 mg/kg body weight has been reported for hexaneamine (not having the 2 methyl branches that DMAA has) (Anonymous, 1954). Based on this information, DMAA would appear to be of moderate acute toxicity.

Acute ingestion of DMAA at a dose of 3 mg/kg body weight (*i.e.*, about 180 to 250 mg or more than [redacted] the maximum recommended serving of DMAA contained in OxyELITE Pro™ and

Jack3d™) by 5 young men was reportedly associated with an increase in systolic blood pressure (possibly by 20 to 30 mmHg) within about 1 hour of dosing. Lesser increases were noted on diastolic blood pressure. A slight decrease in heart rate was also noted (Marsh *et al.*, 1951). There was no indication that any of the test subjects suffered serious adverse reactions or events.

Aliphatic amines, particularly the primary alkyl amines, are generally considered to be non-genotoxic (*i.e.*, do not damage DNA) (Greim *et al.*, 1998).

The available metabolic, pharmacological, and animal data on DMAA however, suggest there is no indication that consumption of low doses (*e.g.*, 10 mg to 20 mg) of DMAA would be harmful in otherwise healthy subjects not taking medication. There exists a published case report of an individual who suffered a cerebral hemorrhage shortly after consuming two "party pills" that each may have contained 278 to 300 mg of DMAA (Gee *et al.*, 2010) for a total of 556 to 600 mg. However, this person may have consumed caffeine, alcohol and/or other recreational drugs at the time they ingested the "party pills". As a result, a definitive causal association between DMAA and this case report cannot be made. In any case, this individual likely consumed 5 to 10-fold more DMAA than would be consumed through use of OxyELITE Pro™ and Jack3d™ at the maximum rate indicated on the label.

Safety of DMAA in USPlabs Formulations:

A number of small-scale clinical trials have been published that pertain to the safety of DMAA as present in 2 of USPlabs' dietary supplement formulations. These include the results of three clinical trials on OxyELITE Pro™ (Farney *et al.*, 2012; McCarthy *et al.*, 2012a,b) and two on Jack3d™ (Bloomer et al., 2012; Farney *et al.*, 2012).

In an acute, double-blind, placebo-controlled, crossover study (McCarthy *et al.*, 2012a), the effects of consumption of OxyELITE Pro™ by 12 exercise-trained subjects on indices of lipolysis and metabolic rate were examined. Six men (24.8±4.3 years of age; 81.1±11.6 kg in weight; and average BMI of 25.5±5.0 kg/m^2) and 6 women (22.8±0.4 years of age; 62.3±12.6 kg in weight; and average BMI of 22.4±2.6 kg/m^2) participated in the study.

Subjects were evaluated through conduct of 2 tests separated by 3 to 4 days. At the beginning of each test, heart rate and blood pressure were measured. A blood and a 5-minute breath sample were also collected. Subjects then ingested 2 capsules of OxyELITE Pro™ or placebo, followed by the collection of the same aforementioned data at 30, 60, 90, and 120 minutes post-ingestion. Blood was only collected at 60 and 120 minutes post-ingestion. During this time subjects were inactive. Subjects recorded food and drink intake during the 24 hours preceding each test. Metabolic rate was determined by indirect calorimetry *via* breath-by-breath collection, and the respiratory exchange ratio was determined from gas collection data and used as a measure of substrate utilization.

Plasma glycerol (area under the curve) increased in the OxyELITE Pro™ group compared to baseline ($p<0.05$) and placebo ($p=0.001$). Serum free fatty acid concentration (area under the curve) also increased in the supplement group ($p<0.0001$). Kilocalorie expenditure also was greater in the OxyELITE Pro™ group ($p=0.005$). Percent change in heart rate was increased in the supplement group over time compared to placebo ($p<0.0001$). In the supplement group,

systolic blood pressure was increased ($p<0.0001$) when compared to placebo controls. The increase was moderate in nature at 10 to 20 mmHg. Less discernible effects were noted on diastolic blood pressure. These results indicated that acute OxyELITE Pro™ supplementation increased markers of lipolysis and metabolic rate and may aid in weight loss over time. Hemodynamic measurements also indicated increased myocardial work. Adverse effects noted in the report included subjects feeling "jittery", "on-edge", "sweaty", and shaky", and subjects having cold sweats, poor sleep quality on the night of treatment, and a racing heart beat. The authors noted that the effects on heart rate and blood pressure should be a source of caution and that subjects with increased blood pressure, especially those with resting blood pressure greater than 140/90 mmHg, should be careful in their use of OxyELITE Pro™, as they would be with any stimulant including caffeine. Depending upon the individual's tolerance to and regular use of caffeine, consumption can acutely increase systolic blood pressure by 3 to 5 mmHg and diastolic blood pressure by 4 to 13 mmHg (Mort and Kruse, 2008). This is in the range of the effect noted for OxyELITE Pro™

The hemodynamic and hematological effects of OxyELITE Pro™ were further explored in a 2-week study conducted with 6 exercise-trained men and women (Farney *et al.*, 2012). Four non-smoking, healthy men and 2 non-smoking, healthy women (combined means of 22.5±1.8 years of age; 65.9±8.6 kg in weight; and average BMI of 21.9±2.1 kg/m^2) were enrolled in the study. Unfortunately, sex-specific data were not provided. In any case, stratification by sex would be of little value given the small number of subjects (*i.e.*, 4 males and 2 females). Subjects were provided with 2 capsules of OxyELITE Pro™ per day for 14 days. No placebo was included in the study. On Days 1 and 15 (pre- and post-intervention), assessments were made pertaining to heart rate, systolic blood pressure, diastolic blood pressure, and rate pressure product. A blood sample was obtained, following which subjects ingested 2 capsules of OxyELITE Pro™. At 30, 60, 90, and 120 minutes post-consumption of the supplement, the above noted cardiovascular parameters were measured. Following testing on Day 1, subjects were provided with OxyELITE Pro™ and instructed to ingest 2 capsules a day, preferably ≥6 hours prior to bedtime. Subjects were asked to maintain their usual diet and physical activity patterns, but refrain from strenuous activity during the 24 hours prior to each test day. Subjects also were asked to rate their overall appetite before and after the intervention.

Four subjects (3 women, 1 man) discontinued the study due to dislike for the supplement or academic/work-related commitments, noting sleeplessness, inability to focus, nausea, headaches, and jitters. These subjects were replaced with 4 additional subjects. The authors also mention that no serious adverse events occurred.

Appetite was noted to be lower from pre- to post-intervention (from 6.3 to 4.3 on the visual analog scale; $p=0.04$). Heart rate measurements taken post-intervention tended to be slightly lower than pre-intervention (*i.e.*, day 15 *vs.* day 1) ($p=0.008$). This correlated with a decrease in the measured rate pressure product. There were no significant differences in systolic or diastolic blood pressure following 2 weeks of supplement use. Systolic blood pressure was higher by about 15 to 16 mmHg at 60, 90, and 120 post-ingestion when compared to pre-ingestion ($p=0.03$) of the supplement at each of days 1 and 15. This effect also translated into slightly higher rate pressure product values 60 to 120 minutes post ingestion on these treatment days. These findings were consistent with the previous study in which myocardial work was increased following acute ingestion of OxyELITE Pro™. However, intake of OxyELITE Pro™

over a 14-day period did not induce any elevation in the cardiovascular parameters examined and did not result in changes in the complete blood count, lipid panel, and metabolic panel. The authors noted that the absence of a placebo control and the small sample size were limitations to this study. As in the acute study, Farney *et al.* (2012) cautions against the use of OxyELITE Pro™ by persons with elevated blood pressure, including those that may be pre-hypertensive (*i.e.*, blood pressure ≥120/80 mmHg), and particularly those with ≥140/90 mmHg.

The findings of Farney *et al.* (2012) were essentially corroborated in an 8-week randomized, double-blind, placebo-controlled study (McCarthy *et al.*, 2012b). Thirty-two exercise-trained subjects (16 men, 16 women) were assessed for efficacy outcomes (body weight, body composition, skin-fold thickness, serum lipids, and appetite) and safety variables (complete blood count, comprehensive metabolic panel, resting heart rate, and blood pressure) in response to consumption of OxyELITE Pro™. Subjects were randomly assigned to consume either a placebo or OxyELITE Pro™ and were instructed to ingest 1 capsule daily for the first 3 days, and increase the dose to 2 capsules per day for the remainder of the study if the single capsule was well-tolerated. Anthropometric measurements and a blood sample were obtained at pre-intervention (Day 1) and post-intervention. The pre-intervention anthropometric and hemodynamic characteristics of the placebo group were not statistically significant from the OxyELITE Pro™ group. However, when comparing pre- and post-intervention values for the OxyELITE Pro™ group, significant decreases were noted in body weight, BMI, waist circumference, waist-to-hip ratio, total body fat percentage, fat mass, fat-free mass, and skin-fold thickness ($p<0.05$).

An increase in resting heart rate was also noted in the treated group (from 63.3±1.9 to 69.4±2.2; $p<0.01$). Based on this the authors concluded that it may be wise for hypertensive individuals to avoid the use of this supplement. A similar caution would pertain to the use of other stimulants such as caffeine. No significant changes in blood pressure measurements were noted following 8-week so treatment with OxyELITE Pro™.

In the OxyELITE Pro™ group, significant increases were noted in total cholesterol, high-density lipoprotein (HDL)-cholesterol, and malondialdehyde, and a decrease was noted in the ratio between low-density lipoprotein (LDL)-cholesterol:HDL-cholesterol when comparing pre- and post-intervention values ($p<0.05$). Statistically significant changes in red blood cells, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets, neutrophils, and monocytes were noted, however, all were minimal in nature and of no biological significance. There were no adverse changes in serum concentrations of AST, ALT, GGT, alkaline phosphatase or bilirubin. These parameters are indices of hepatobiliary function.

With respect to Jack3d™, there exists two published clinical trials, that being the studies of Farney *et al.* (2012) described above which also assessed OxyELITE Pro™, and a 10 week clinical trial of Jack3d™ (Bloomer *et al.*, 2012).

Farney *et al.* (2012) explored the potential hemodynamic and hematological effects of Jack3d™ in a 2-week study with healthy active non-smoking adults. Seven men (24.9±4.2 years of age; 83.8±9.4 kg in weight; and average BMI of 26.5±3.3) consumed 2 servings of Jack3d™ (powder mixed in water) for 14 consecutive days. The design and conduct of this study was discussed above. After 14 days of intake of Jack3d™, no effects on resting hemodynamic variables could be detected. This would suggest that the consumption of Jack3d™ for 2 weeks does not lead to

permanent alterations of cardiovascular function. Shortly after (30 to 120 minutes) consumption of Jack3d™ there was a tendency for an approximately 5 to 15% increase in systolic and diastolic blood pressure, and in the calculated rate pressure product, although these values failed to achieve statistical significance. Analysis of the hematological data revealed no adverse effects on any of the parameters measured. Blood glucose was higher (94.6±2.0 mg/dL compared 86.4±2.3 mg/dL; p=0.02) after 2 weeks of consumption of Jack3d™, however, the authors did not ascribe any clinical significance to this finding. These data indicate that beyond transitory acute effects on cardiovascular variables, no overt toxicity is associated with the consumption of Jack3d™ for a period of 2 weeks. The study authors did note that the study was limited by the use of few subjects. Farney *et al.* (2012) caution against the use of Jack3d™ by persons with elevated blood pressure, including those who may be pre-hypertensive (*i.e.*, blood pressure ≥120/80 mmHg).

In the 10-week, randomized, placebo-controlled study (Bloomer *et al.*, 2012), 25 healthy men were randomly assigned to either a placebo (n=13) or treated group (n=12) (1 to 3 servings of Jack3d™ per day on "workout" days, 30 minutes prior to an exercise session) (n=12) for a period of 10 weeks. Resting blood pressure and heart rate were measured, and blood samples were collected, prior to the start and at the end of the 10-week intervention period. Subjects were asked to maintain their usual physical activity and dietary habits during the 10-week period. Food and drink consumed the week before each lab session was recorded by each subject.

No subject reported any adverse events that could be ascribed to either Jack3d™ or placebo treatment. After 10 weeks of study, no statistically significant differences were noted between the placebo and treatment groups with respect to blood pressure (p>0.05), although systolic blood pressure increased approximately 6 mmHg, and diastolic blood pressure decreased approximately 4 mmHg, with the use of the supplement. Heart rate decreased slightly (3 to 6 bpm), but significantly, in both the treated and placebo groups. Rate pressure product was unaffected by 10 weeks of treatment with Jack3d™. Analyses of the blood samples revealed several statistically significant changes (*e.g.*, creatinine increased from pre- to post-intervention; alkaline phosphatase decreased from pre- to post-intervention). There were no changes in serum enzymes (*e.g.*, AST, ALT, and GGT) indicative of altered liver function. The authors concluded that these data showed that the consumption of a dietary supplement containing DMAA for a period of 10 weeks did not result in a statistically significant increase in resting blood pressure or heart rate in this sample of healthy men. A non-significant increase in mean systolic blood pressure was noted, hence, it was considered wise for those with hypertension to not use dietary supplements containing DMAA.

Safety of DMAA in Combination with Caffeine:

The same group of researchers who studied USPlab's two formulations has also conducted human trials with specific combinations of DMAA and caffeine, either alone or in combination (Bloomer *et al.*, 2011a,b).

In a double-blind, randomized, crossover design acute study, 10 young healthy, exercised-trained, non-smoking men (n=5, age 26±5 yrs, weight 88±11 kg) and women (n=5, age 23±3 yrs, weight 73±17 kg) ingested 1 of 5 different combinations of caffeine and DMAA on different days (Bloomer *et al.*, 2011a). The following were ingested as a single dose after a 10-

hour overnight fast: 250 mg caffeine, 50 mg DMAA, 75 mg DMAA, 250 mg caffeine + 50 DMAA, and 250 mg caffeine + 75 mg DMAA (Bloomer *et al.*, 2011a). There was no placebo group. The doses in this study were higher than the doses from single servings of either OxyELITE Pro™ or Jack3d™. Also, the 75 mg dose was greater than the maximum daily recommended dose of DMAA that could be achieved by use of these supplements according to label instructions.

Heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), and rate pressure product (RPP) were measured pre-ingestion and at 30, 60, 90, and 120 minutes post-ingestion. Subjects were instructed not to exercise 24 hours prior to visiting the laboratory. Plasma norepinephrine (NE) and epinephrine (EPI) were measured pre-ingestion and at 60 and 120 minutes post-ingestion. Following ingestion of the various combinations of DMAA and caffeine, there was no indication of an increase in heart rate. A slight tendency for a decrease in heart rate was actually noted in most of the groups. In contrast, systolic blood pressure was found to increase in a dose-dependent manner with the greatest increases in the 250 mg caffeine + 75 mg DMAA group (*i.e.*, systolic blood pressure of 143±5 mmHg 60 minutes post-ingestion *vs.* 119±3 mmHg pre-ingestion). Smaller increases in systolic blood pressure were noted with 250 mg caffeine + 50 mg DMAA (*i.e.*, 133±4 mmHg at 90 minutes post-ingestion *vs.* 119±4 mmHg) and 50 mg DMAA alone (*i.e.*, 128±4 mmHg 90 minutes post-ingestion *vs.* 121±4 mmHg). Fifty mf DMAA alone had a similar effect as 250 mg caffeine alone (*i.e.*, 123±5 mmHg 120 minutes post-ingestion *vs.* 117±3 mmHg). Rate pressure product was increased in the high dose DMAA and caffeine group compared to the caffeine alone group. Diastolic blood pressure tended to increase by about 5 to 10 mmHg in all of the groups regardless of dose. Plasma NE and EPI were relatively unaffected by treatment. The authors concluded on the basis of their data that acute dosing with DMAA alone and in combination with caffeine results in an increase in systolic and diastolic blood pressure, and in the rate pressure product, without a concomitant increase in heart rate. The authors speculated that some of these effects may be associated with vasoconstricting activity of DMAA. They also noted the relatively greater potency of 75 mg DMAA and 75 mg DMAA and 250 mg caffeine (as a single dose) compared to the other treatment groups. The 50 mg dose of DMAA reportedly produced similar effects as 250 mg of caffeine.

In another, double-blind, cross-over design study (Bloomer *et al.*, 2011b), 6 exercise-trained (regular runners) subjects (avg. age 21.9 years, weight 67.6 kg) of each sex consumed either a placebo, 4 mg/kg body weight of caffeine (~200 to 300 mg), 1 mg/kg/body weight DMAA (~50 to 70 mg), or a combination of these treatments, 1 hour prior to physical activity in the form of a 10 km run. All subjects were asked to complete the run as fast as possible. Each subject completed the run using each of the treatment conditions. The runs were each separated by 1 week. Blood was collected prior to the run and 5 and 30 minutes after the run and analyzed for markers of lipolysis and oxidative stress (*i.e.*, glycerol, free fatty acids, malondialdehyde, nitrate/nitrite). At these times, systolic and diastolic blood pressure was also measured and rate pressure product calculated. Heart rate was measured every 2 km during the run.

The consumption of caffeine or DMAA alone and in combination had no statistically effects on run times, subjects' perceived amount of exertion or mood or sense of vigor. Heart rate during the run was unaffected by either caffeine or DMAA, alone or in combination. Blood concentrations of glycerol and free fatty acids were increased following the run in all treatment groups. Ingestion of DMAA alone had a greater effect on these parameters compared to

caffeine alone or in combination with DMAA. Prior to the run, systolic blood pressure was found to be increased (15 to 30 mmHg) in subjects 60 minutes following consumption of either DMAA or caffeine alone relative to the placebo and caffeine and DMAA in combination treatments. This difference appeared to persist through to 30 minute post exercise. The similar values noted between the caffeine and DMAA in combination treatment *vs.* placebo was in contrast to the findings of Bloomer *et al.* (2011a). No biologically significant differences between the treatment groups were noted with respect to diastolic blood pressure.

Discussion and Conclusions with Respect to the Safety of DMAA in USPlabs Formulations

There are no long-term animal data to support the safety of DMAA for use in dietary supplement products. DMAA is expected to be of moderate acute toxicity. It is known to have both α and β adrenergic activity. The limited metabolic, pharmacological, and animal data on DMAA provide no direct indication that consumption of low doses (*e.g.*, 10 mg to 20 mg) of DMAA would be harmful in otherwise healthy subjects not on medication.

The most relevant safety data pertaining to the use of DMAA in dietary supplements comes from the results of small-scale, short- and medium-term clinical trials in healthy subjects who consumed either dietary supplements containing DMAA, namely OxyELITE Pro™ (Farney *et al.*, 2012; McCarthy *et al.*, 2012a,b;) and Jack3d™ (Bloomer *et al.*, 2012; Farney *et al.*, 2012;), or DMAA either alone or in combination with caffeine (Bloomer *et al.*, 2011a,b). It should be noted that each of these clinical trials were limited by the inclusion of small numbers of subjects, lack of stratification by sex, and study only in healthy subjects. Doses of DMAA in these clinical trials ranged from 20 to 75 mg/day, often in combination with caffeine (250 to 375 mg/day) and/or other constituents.

The results of the clinical trials indicate that the main effect of DMAA when administered alone, in combination with caffeine, and or as part of multi-component dietary supplement products, is a moderate elevation (10-20 mmHg) in systolic blood pressure within 30 to 60 minutes of ingestion (Bloomer *et al.*, 2011a,b; Farney *et al.*, 2012; Bloomer *et al.*, 2012; McCarthy *et al.*, 2012a). The effect may last for 2 or more hours and appeared dose-dependent. A 50 mg dose of DMAA reportedly produces similar effects as 250 mg of caffeine (Bloomer *et al.*, 2011a). Also, as stated previously, consumption of caffeine from coffee can acutely increase systolic blood pressure by 3 to 5 mmHg and diastolic blood pressure by 4 to 13 mmHg (Mort and Kruse, 2008).

There is no indication that longer-term use results in an increase in resting systolic blood pressure. The study authors cautioned against the use of DMAA or supplements containing DMAA by persons with elevated blood pressure (*i.e.*, >120/80 mmHg).

There were no reports of any serious adverse events in the human trials. Some subjects noted feeling jittery, sweaty, on edge, or euphoric in response to treatment with DMAA alone or in combination with caffeine, and/or with DMAA as part of dietary supplement products. These responses are typical and often noted with consumption of stimulants. There were no indications of potential target organ toxicity based on the results of serum biochemistry and haematological analyses (Bloomer *et al.*, 2012; Farney *et al.*, 2012; McCarthy *et al.*, 2012b).

In regards to safety of DMAA, a point of consideration is the pharmacological activity (*i.e.*, vasoconstriction) of this substance. The available clinical trial data do indicate a mild, transient

and acute blood pressure increasing effect of DMAA, and its associated supplement products. Use of DMAA would be contraindicated in persons with hypertension or those with marginal hypertension who may use the product under conditions which could further elevate blood pressure (*i.e.*, strenuous exercise). The limited data indicate that following exercise DMAA increases systolic blood pressure moderately in trained individuals (Bloomer *et al.*, 2011b).

Based on information provided by USPlabs, there have been inquiries regarding a possible association between DMAA consumption and occurrences of liver and/or renal toxicity, seizures, and rhabdomyolysis. Details of these case reports were not available. It should be noted that with any "case-report", it is not possible to conclusively disprove such associations (*i.e.*, it is not scientifically possible to prove a "negative"). However, based on the results of the clinical trials, such effects would appear unlikely to occur at DMAA doses associated with the recommended servings of OxyELITE Pro™ and Jack3d™. Biochemical data from the clinical trials show no adverse effect on hepatorenal function and no adverse events suggestive of seizure activity or rhabdomyolysis were observed.

In the case of the DMAA-containing products OxyELITE Pro™ and Jack3d™, it is our understanding that the label states or indicates: a maximum dose limit of 1 to 3 servings, the need for mandatory clearance from a physician, and a recommendation for the incorporation of an initial tolerance dosing period (this is important for first time users so as to detect any cardiovascular sensitivities to the combination of caffeine and DMAA. The label also provides that the maximum duration of continuous use should be 5 days per week (or only on "workout" days in the case of Jack3d™) for 8 weeks, and that the individual be healthy and to avoid using the products if they have various medical conditions, taking certain medications, other dietary supplements or wishing to avoid caffeine. The servings should also not be consumed less than 4 hours apart. Based on these labeling conditions, it is unlikely that DMAA consumption from these products would cause adverse effects when used as directed by the appropriate population.

Overall, under the intended conditions of use, and adherence to the labeling instructions as discussed above, it is concluded that it is unlikely that adverse effects would occur from the consumption of DMAA as part of the dietary supplements OxyELITE Pro™ or Jack3d™. Use of DMAA, either alone or as part of dietary supplement products, at excessive doses, or by persons with certain medical conditions and/or sensitivities, could theoretically lead to adverse cardiovascular events. This though is true with respect to the consumption of any substances, including caffeine, alcohol, nicotine, cold medications, *etc.*, that have pharmacological activity on the cardiovascular system.

Occurrence of DMAA in the Geranium Plant (*Pelargonium graveolens*) and Essential Oil

From a safety standpoint, whether or not DMAA is synthesized or extracted from natural sources is of no consequence. However, questions have been raised as to whether DMAA is naturally occurring in geranium. Initially the presence of DMAA in geranium oil was largely based on the results of an analytical study conducted by researchers at Guizhou Institute of Technology in Guizhou China (Ping *et al.*, 1996). Other, more recently published studies (Jain *et al.*, 2001; Rao *et al.*, 2002; Shellie and Marriott, 2003; Jalali-Heravi *et al.*, 2006; Fayed, 2009), however, have not identified the presence of DMAA in either the essential oil or in the plant tissue. This has led to some controversy regarding the occurrence of DMAA in geranium oil and plant tissue.

First, it is worth reviewing in some detail the results of the Ping *et al.* (1996) study. Based on a translated version of the study provided to Intertek Cantox, these researchers assessed air-dried samples of fresh *Pelargonium graveolens* stems and leaves. The stems and leaves were reportedly cut and subject to steam distillation to yield a dark-yellow volatile oil (0.15% w/w). The oil was then analyzed by gas chromatography (GC) coupled with mass spectroscopy (MS) (*i.e.*, GC-MS).

Analysis of the MS spectra was reported to result in the identification of 31 chemical components. The relative quantity of each component present was estimated through the "surface chemical method". With this method Ping *et al.* (1996) accounted for 95.07% of the constituents of the oil extract.

The identity and specific concentrations of the 31 chemicals identified in the oil were reported by Ping *et al.* (1996) in a tabular format, documenting chemical name (English), chemical name (Chinese), chemical formula, molecular weight, and concentration detected. A copy of this table as provided by USPlabs is shown in Figure 2.

Figure 2 **Table From Original Ping *et al.* (1996) Study**

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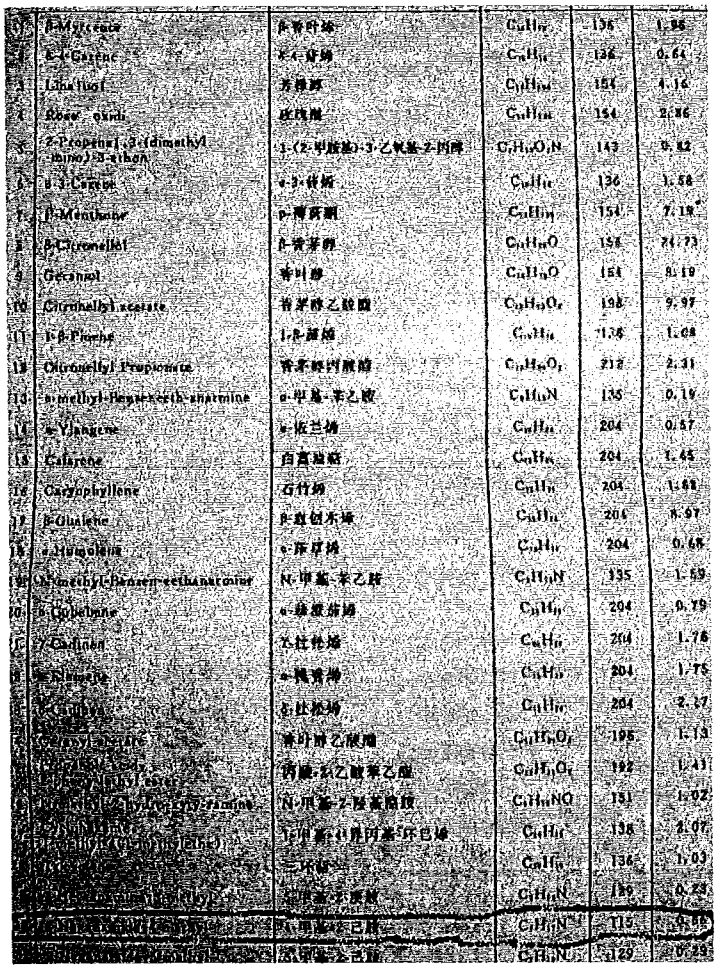

7	β-Myrcene	β-香叶烯	$C_{10}H_{16}$	136	1.95
8	E-β-Ocimene	反-β-罗勒烯	$C_{10}H_{16}$	136	0.64
9	Linalool	芳樟醇	$C_{10}H_{18}O$	154	4.16
10	Rose oxide	玫瑰醚	$C_{10}H_{18}O$	154	2.86
11	2-Propenal,3-(dimethylamino)-2-ethyl-	3-(2-甲胺基)-3-乙氧基-2-丙醛	$C_7H_{13}O_2N$	143	9.82
12	δ-3-Carene	δ-3-蒈烯	$C_{10}H_{16}$	136	1.58
13	p-Menthone	p-薄荷酮	$C_{10}H_{18}O$	154	7.18
14	β-Citronellol	β-香茅醇	$C_{10}H_{20}O$	156	74.73
15	Geranal	香叶醛	$C_{10}H_{16}O$	152	8.19
16	Citronellyl acetate	香茅醇乙酸酯	$C_{12}H_{22}O_2$	198	9.97
17	1-β-Pinene	1-β-蒎烯	$C_{10}H_{16}$	136	1.08
18	Citronellyl Propionate	香茅醇丙酸酯	$C_{13}H_{24}O_2$	212	2.31
19	α-methyl-Hexa-ketih-ehetmine	α-甲基-苄乙胺	$C_9H_{13}N$	135	0.19
20	α-Ylangene	α-依兰烯	$C_{15}H_{24}$	204	0.57
21	Calarene	白菖蒲烯	$C_{15}H_{24}$	204	1.45
22	Caryophyllene	石竹烯	$C_{15}H_{24}$	204	1.68
23	β-Guaiene	β-愈创木烯	$C_{15}H_{24}$	204	8.97
24	α-Humulene	α-葎草烯	$C_{15}H_{24}$	204	0.68
25	N-methyl-Benzen-ethanamine	N-甲基-苄乙胺	$C_9H_{13}N$	135	1.59
26	δ-Guaiene	α-愈创烯	$C_{15}H_{24}$	204	0.79
27	γ-Cadinen	γ-杜松烯	$C_{15}H_{24}$	204	1.76
28	δ-Cadinene	α-杜松烯	$C_{15}H_{24}$	204	1.75
29	δ-Cadinene	δ-杜松烯	$C_{15}H_{24}$	204	2.17
30		2-己酰胺-4-甲基	$C_7H_{17}NO$	115	1.13
31		香叶醇乙酸酯	$C_{12}H_{20}O_2$	196	1.41
32		香茅醇乙酸苯乙酯	$C_{20}H_{30}O_2$	302	1.02
33		N-甲基-2-羟基烟碱	$C_{11}H_{14}NO$	151	2.07
34		1-甲氧基-4-丙烯基苯	$C_{10}H_{12}$	136	1.03
35		三环烯	$C_{10}H_{16}$	136	0.73
36		苄基乙胺	$C_9H_{13}N$	135	0.48
37		苯甲醛	C_7H_6O	106	0.29
38		苯甲腈	C_7H_5N	115	0.48
39		苯乙腈	C_8H_7N	129	0.29

In the table presented in Figure 2, compound 30 is circled. It has been identified in the table as 2-hexaneamide-4-methyl. However, a review of that row of the table clearly indicates that the chemical referred to was 2-hexanamine-4-methyl (*i.e.*, DMAA). Both the chemical formula (*i.e.*, $C_7H_{17}N$) and the molecular weight (*i.e.*, 115) denote the amine, not the amide. If the chemical were the amide, it would contain an oxygen atom, which is clearly not present in the chemical

formula. In all probability the authors referred to the amine, however, the amide term was introduced into the table likely as a result of English not being their primary language or the primary language of the journal editors and/or referees. This interpretation of the inconsistency in the table is further bolstered by the fact that in the Chinese name, the character used is "胺". This denotes the amine, not the amide which would have been "酰胺". Given the foregoing, it is evident that based on the methodology employed (GC-MS) and the specimen analyzed (steam distillation oil extract from *Pelargonium graveolens* stem and leaves, obtained from the Rongjiang Region of Guizhou Province) that Ping *et al.* (1996) did in fact identify the presence of DMAA. Ping *et al.* (1996) reported the relative concentration present to be 0.66%.

To further document the presence of DMAA in geranium oil extract and plant tissues, USPlabs provided Intertek Cantox with the results of an analytical study conducted by a well respected testing laboratory. To the knowledge of Intertek Cantox, these results are in the process of being published.

In this study, geranium oil samples sourced from China were tested by liquid chromatography and mass spectroscopy (LC-MS-MS) specifically for the presence of DMAA. The method developed by the analytical laboratory was subject to a method validation protocol as prescribed by USP 33 <1225>. This validation method assessed the analytical test's capabilities and adherence to standards with respect to linearity and range, accuracy, precision, detection limit and quantification limit, and specificity. The results of these procedures demonstrated that the testing methodology (LC-MS-MS) was accurate, precise, and specific for the determination of DMAA in geranium oil. The initial sample of geranium oil tested in the validation assay was found not to contain detectable quantities of DMAA.

The subsequent analytical testing of another sample of geranium oil sourced from different lot was reported by the testing laboratory to contain DMAA. This analyte was assessed using the validated analytical methodology. Testing of samples of geranium oil by the validated method yielded DMAA concentrations in the range of <1 to greater >10,000 ppb. The amounts detected in the geranium oil samples using the validated methodology were orders of magnitude lower than the levels (*i.e.*, 0.66% or 6,600,000 ppb) reported in the original Ping *et al.* (1996) study. Additional study of geranium plant tissues revealed the presence of DMAA at concentrations of 10s to 100s of ppb.

The achieved limits of detection and/or quantification associated with different methods of analyses may in part explain the lack of finding of DMAA in other publications reporting on the constituents present in geranium oil (Jain *et al.*, 2001; Rao *et al.*, 2002; Shellie and Marriott, 2003; Jalali-Heravi *et al.*, 2006; Fayed, 2009). In these publications, each of which employed GC-MS type methodologies in their analysis, the limits of detection/quantification were not specifically stated, however, the "peak areas" were reported to only the tenths or 1/100s of percent. In those publications which did not detect DMAA, its possible presence cannot be ruled out given that not all of the samples' constituents were specifically identified.

It is critically important that one understand that the Jain *et al.* (2001), Rao *et al.* (2002), Shellie and Marriott (2003), and Jalali-Heravi *et al.* (2006) studies were conducted to identify the major components (*i.e.*, those comprising 0.01% to 0.1% [*i.e.*, 100,000 to 1,000,000 ppb] or more) of the essential oil from geranium plants from specific regions of the world. These studies were not designed to assess the presence of compounds at trace levels (*i.e.*, ppb range). The

assessment of such compounds requires the development of specific validated analytical methodologies that can reliably detect the compounds of interest. Such methods were used to detect DMAA in a sample of geranium oil from China. Since the purpose of the studies which did not report the presence of DMAA in geranium oil was not to assess specific trace constituents, it may be inappropriate to cite these as examples to indicate that such trace constituents are not there.

That DMAA would not be present in every sample of geranium oil and/or plant tissue is not unexpected. This appears to be borne out in the analyses conducted recently for USPlabs whereby the geranium oil sample used in the validation protocol had no detectable amounts of DMAA while another sample contained >100 ppb DMAA. Moreover, it is apparent that geranium oils from plants sourced from differing locales show qualitative and/or quantitative differences with respect to their constituent makeup (Jain *et al.*, 2001; Rao *et al.*, 2002; Babu and Kaul, 2005; Jalali-Heravi *et al.*, 2006; Fayed, 2009). Significant quantitative and/or qualitative variance in the constituents, including major constituents, of herbal plants is to be expected (Canigueral *et al.*, 2008; Chizzola, 2010; Medini *et al.*, 2010; Sahoo *et al.*, 2010; Smith *et al.*, 2010). These variances occur as a result of differing geographical location, weather conditions/climate, harvesting practices, soil, photoperiod, *etc.*

Analysis of the oil extract form geranium plants of differing geographic origin, as would be expected, shows significant differences in the concentrations, and even the presence, of the various compounds found in the essential oil at concentrations of 0.01% to 0.1% or more (Rao *et al.*, 1995; Jain *et al.*, 2001; Rao, 2002; Shellie and Marriott, 2003; Babu and Kaul, 2005; Verma *et al.*, 2010). Given that there are both qualitative and quantitative differences in the "major" constituents of geranium plants sourced from different locations throughout the world, it is not surprising that such differences would also exist with respect to trace constituents such as DMAA.

In summary, it is concluded that the analytical study provides definitive identification of trace levels (ppb) of DMAA in specific samples of geranium oil and geranium plant tissues. DMAA may not be identified or detected in some geranium oils samples due to limitations of analytical methodologies employed and/or variations in the chemical makeup of plants sourced from different locales and/or suppliers.

Sincerely,

Barry Lynch, B.Sc., DABT
Associate Director

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Intertek

CANTOX
HEALTH SCIENCES INTERNATIONAL

Memorandum

To: To Whomever It May Concern

From: Barry Lynch

Date: September 6, 2011

The question has been raised as to whether a compound which USPlabs utilizes in some sports nutrition supplements, 1,3 Dimethylamylamine ("DMAA") occurs in nature.

Although published data already exists (Ping *et al.*, 1996) which indicate that DMAA does occur in geranium oil derived from geranium (*Pelargonium graveolens*) plants gathered in specific region(s) of China, translational and grammatical errors in this report have led some to question the validity of the data. Consequently, USPlabs undertook a scientific investigation to verify that DMAA does in fact occur in the geranium plant (as well as its oil) and at what concentrations. The data that USPlabs has obtained from two independent and highly respected analytical chemistry laboratories, utilizing advanced validated analytical instrumentation and methods, corroborate the original data published by Ping et al, and further demonstrate the occurrence of DMAA in the geranium plant, *Pelargonium graveolens*, and its edible oil.

USPlabs has also had their formulations containing this compound evaluated in human clinical trials. No serious adverse events have been encountered and the formulations containing DMAA have been well-tolerated.

USPlabs is currently in the process of having these data prepared for and submitted to peer-reviewed journals for publication. As you are well aware, this process can take several months to over a year from the time of submission to publication. Because highly respected peer-reviewed journals have strict policies which prohibit the dissemination / publication of data prior to publication in the journal, USPlabs is unable to release data prior to publication.

Barry Lynch, B.Sc., DABT, ERT
Associate Director
Cantox Health Sciences International
An Intertek Company

Analytical Chemistry Insights

ORIGINAL RESEARCH

Analysis and Confirmation of 1,3-DMAA and 1,4-DMAA in Geranium Plants Using High Performance Liquid Chromatography with Tandem Mass Spectrometry at ng/g Concentrations

Heather L. Fleming, Patricia L. Ranaivo and Paul S. Simone

Department of Chemistry, The University of Memphis, Memphis, TN, USA.
Corresponding author email: psimone@memphis.edu

Abstract: 1,3-Dimethylamylamine (1,3-DMAA) is a stimulant commercially sold in a variety of dietary supplements as a chemical species derived from geranium plants (Pelargonium graveolens). Whether 1,3-DMAA naturally occurs in geranium plants or other dietary ingredients, it has important regulatory and commercial ramifications. However, the analysis of 1,3-DMAA in geranium plants is not trivial due to low concentrations and a complex environmental matrix, requiring high selectivity and sensitivity. An extraction method combined with high performance liquid chromatography and tandem mass spectrometry is used to determine 1,3-DMAA and 1,4-dimethylamylamine (1,4-DMAA) concentrations in geranium plants with both external calibration and standard addition method. Samples from the Changzhou, Kunming, and Guiyang regions of China during both winter and summer were analyzed for 1,3-DMAA and 1,4-DMAA. The diastereomer ratios of the 1,3-DMAA stereoisomers of a racemic standard and the extracted plant were also quantified.

Keywords: DMAA, geranium, natural product analysis, HPLC, mass spectrometry

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Introduction

There has been significant discussion of 1,3-dimethylamylamine (1,3-DMAA) in the literature concerning the presence of 1,3-DMAA in geranium plants (*Pelargonium graveolens*).[1–6] 1,3-DMAA, also known as 4-methyl-2-hexaneamine (MHA), 1,3-dimethylpentylamine, or 2-amino-4-methylhexane can be labeled as geranium extract in dietary supplements. Confirming the presence or absence of 1,3-DMAA as a natural product in geranium plants has important regulatory and commercial consequences for many dietary supplement companies.[7]

The chemical properties and concentrations of 1,3-DMAA and the associated matrix do not allow for simpler LC detection methods (UV-visible absorption or refractive index). Typically, GC-MS analysis requires derivatization to a higher molecular weight to increase boiling point and retention time. The geranium oil and plant matrix are sufficiently complex that most universal detectors, such as refractive index and flame ionization detectors, are likely to encounter significant matrix interferences. Thus, research and analytical effort for 1,3-DMAA analysis has focused on GC-MS[1,3–5] and LC-MS/MS [1,2,4–6] analysis protocols for matrices, such as urine, geranium oil extracts and geranium plants.

The World Anti-Doping Agency requires that compounds with chemical structure and biological activity similar to banned substances must be analyzed by anti-doping laboratories. 1,3-DMAA and 2-aminoheptane (a banned stimulant) have similar chemical structures and physiological stimulant effects (Fig. 1). The laboratory of Saudan[1] developed a high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for detection of 1,3-DMAA in urine samples. The method was calibrated over the range of 50 to 700 ng/mL with excellent intraday precision and accuracy of less than 6%. The results from the Saudan laboratory found that 1,3-DMAA could be detected in urine samples up to 105 hours after administration of a 40 mg dose.

Subsequent research by Vorce et al[2] used LC-MS/MS to confirm 1,3-DMAA as the cause of false positives in amphetamine screening kits used by the United States Department of Defense drug screening laboratories. 1,3-DMAA was suspected due to its inclusion in bodybuilding energy supplements available over the counter. Vorce et al reported that 1,3-DMAA would cause false positives at urine concentrations above 6.0 mg/L and confirmed the presence of 1,3-DMAA concentrations over the 6.0 mg/L limit in 92.3% of the false positive results for amphetamines.

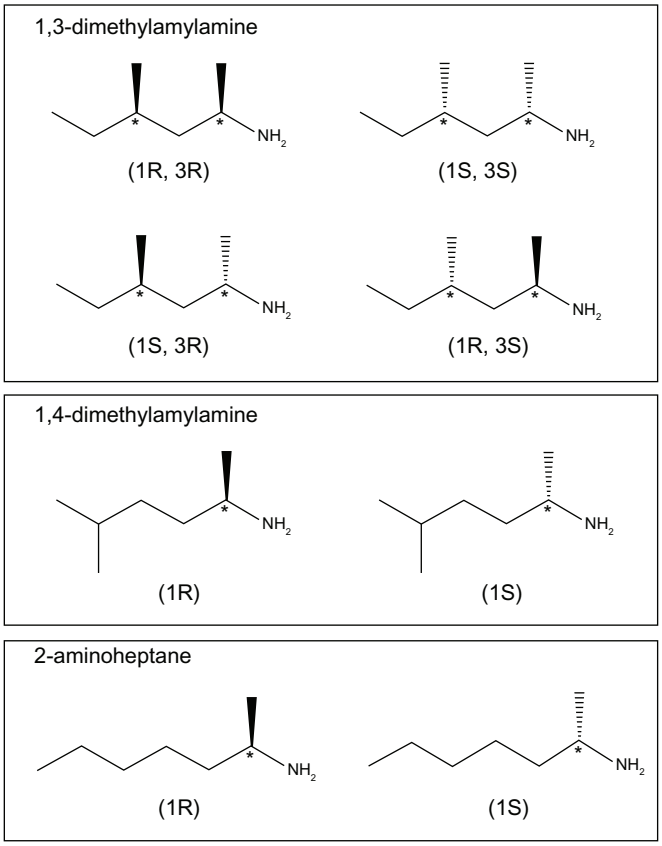

Figure 1. Chemical structures of the stereoisomers of 1,3-DMAA, 1,4-DMAA, and 2-aminoheptane with stereogenic carbons labeled (*) and their respective *(R,S)* configurations.

The laboratory of Lisi[3] conducted an analysis of five geranium oils which had origins in France, Egypt, and New Zealand. The geranium oils were analyzed using a derivatization and extraction procedure for 1,3-DMAA. None of these samples were reported to have 1,3-DMAA, but no limit of detection (LOD) was reported for the method. Supplements containing 1,3-DMAA were then administered and tested in a urine excretion study using a GC with a nitrogen-phosphorous detector. The results showed that 1,3-DMAA is excreted for at least 29 hours in agreement with a previous report.[1]

The research team of ElSohly et al[4] used GC-MS, LC-MS/MS, and high resolution ultra-performance LC with quadrupole-time of flight-MS (UPLC-QTOF-MS) to analyze geranium oils and leaves from India as well as geranium leaves, stems, and freshly extracted

oil from plants grown in Oxford, Mississippi. The GC-MS and LC-MS/MS-based methods used similar extraction procedures with a reported extraction efficiency of 35% (which is relatively low). However, the extraction was shown to have excellent accuracy (75%) and precision (less than 5%) on the control sample using GC-MS analysis. The limits of detection for the GC-MS, LC-MS/MS, and UPLC-QTOF-MS were 0.1 ppm, 2.5 ppb, and 10 ppb, respectively. The GC-MS analysis of the 0.1 ppm spikes of 1,3-DMAA in the geranium oil clearly showed the characteristic double peaks of the 1,3-DMAA diastereomer pairs. The authenticated geranium plant material showed a similar pattern to the spiked geranium oil, whereas the negative geranium oil and authenticated geranium oil did not. The GC-MS chromatograms of the authenticated geranium plant material suggested the presence of the 1,3-DMAA. However, the two more sensitive LC-MS/MS methods did not detect 1,3-DMAA in any of the samples analyzed. The LC-based methods do not exhibit the characteristic diastereomer double peak—possibly due to the chromatographic separation conditions.[2,5,6]

Zhang et al[5] recently reported the analysis of eight different geranium oils, four from China and four from Egypt, and analysis of thirteen dietary supplements containing 1,3-DMAA. The goal of their paper was to determine whether the 1,3-DMAA in dietary supplements had synthetic or natural origins. The supplements were analyzed using GC-FID analysis with a chiral column. The 1,3-DMAA in the standards and supplements were derivatized by pentafluoropropionic anhydride (PFPA). The derivatized stereoisomer separation of 1,3-DMAA by GC-FID was excellent, showing all four stereoisomers present. The GC-FID analysis protocol did not have an LOD reported; however, the calibration curve range was 0.2 to 0.8 mg/mL of 1,3-DMAA. The dietary supplements were reported to contain the same stereoisomer ratios as the synthetic standards.

Zhang et al then used two LC-MS-based methods to analyze the geranium oils for 1,3-DMAA.[5] The LOD of the linear ion trap method (HPLC-ESI-LIT) was 50 ppb and the LOD of the triple quadrupole instrument (HPLC-ESI-QQQ) was 10 ppb for derivatized 1,3-DMAA. The HPLC-ESI-LIT used a chiral-phase HPLC separation column. The HPLC-ESI-QQQ used a standard C18 separation phase. In both methods, 1,3-DMAA was not detected above the LOD, and both lacked the characteristic diastereomer double peak as expected (both possibly due to chromatographic separation choices).

Finally, the research team of Li et al[6] developed an extraction and LC-MS/MS-based method for the analysis of 1,3-DMAA and 1,4-DMAA in geranium plants and oils (three distinct samples of each). The method validation was detailed and conducted according to United States Pharmacopeia guidelines. The traditional instrument LOD[8] reported was 1 to 2 pg/g with a reported method quantification limit (LOQ) of 1 to 2 ng/g in the geranium sample. Li reported concentrations of 1,3-DMAA and 1,4-DMAA as present in three samples of geranium plants ranging from 13 to 365 ng/g and 3 to 35.3 ng/g, respectively. In the geranium oil, Li et al reported all three samples contained 1,3-DMAA ranging from 167 to 13,271 ng/g. In the sample containing 13,271 ng/g of 1,3-DMAA, 1,4-DMAA was detected at 220 ng/g. The other two geranium oil samples did not contain 1,4-DMAA above the LOD.

The research and sample analysis presented here used an adapted extraction and LC-MS-MS analysis[6,9] to analyze both 1,3-DMAA and 1,4-DMAA in geranium plants. Linearity, method detection limit (MDL), accuracy, and precision studies were carried out followed by analysis of geranium plants from 3 distinct regions in China (Changzhou, Guiyang, and Kunming) during winter and summer months. An improved analysis protocol was developed that used standard addition analysis to re-analyze samples and confirm the reported concentrations of 1,3-DMAA and 1,4-DMAA. One of the Changzhou, China, samples was analyzed by another laboratory,[6] and to the best of the authors' knowledge, this represents the first inter-laboratory analysis and confirmation of 1,3-DMAA in an identical geranium sample. Additionally, the diastereomer ratio of 1,3-DMAA in geranium plants was measured and compared with synthetic standards and previously reported research.[5]

Experimental

Chemicals and reagents

All chemicals and reagents have a purity of 97% or greater. All standards and eluent were prepared in reagent-grade water with a resistivity of 18.2 MΩ · cm produced by a Barnstead e-pure four cartridge system.

Glassware was cleaned with concentrated detergent and rinsed with reagent-grade water three times. 1,3-DMAA was purchased from 2A PharmaChem USA (purity confirmed by NMR) and 1,4-DMAA was purchased from Sigma-Aldrich. LC-MS grade acetonitrile and formic acid, HPLC grade ethanol and hexane, and ACS Certified Plus concentrated hydrochloric acid were purchased from Fisher Scientific.

Standard preparation

A combined stock solution was first prepared containing both standards (1,3-DMAA and 1,4-DMAA) with a concentration of 1000 mg/L each in ethanol. An intermediate standard solution is then diluted from the stock to prepare a standard with a concentration of 1000 µg/L in 0.5 N HCl for both 1,3-DMAA and 1,4-DMAA. Two external calibration curves were prepared for each analysis due to the unknown concentrations of 1,3-DMAA. The low range calibration was 1 to 20 µg/L, and the high range calibration was 3 to 100 µg/L. The standard addition spikes curves were prepared by analyzing sample spikes of 1,3-DMAA and 1,4-DMAA at 15.0 µg/L and 25.0 µg/L for each sample.

Sample preparation

Preliminary homogenization and extraction protocol

The preliminary extraction method was adapted from a standard analysis method.[9] The method was scaled from 200 g to 50 g of geranium plant for analysis, and each subsequent step was appropriately scaled by a factor of four. The geranium plants were first cut into pieces having a mass ranging from 40 to 50 g and subsequently placed into a blender. A solution of 15 mL of 0.5 N HCl was added to extract the 1,3-DMAA and 1,4-DMAA analytes present in the plants. The mixture was homogenized at high speed for two minutes, filtered, and re-extracted with 7.5 mL of 0.5 N HCl. Both extracts were combined and diluted to a final volume of 25.00 mL. The solution was then sonicated, filtered, and analyzed by LC-MS/MS. A blank (no geranium plant) and spiked samples containing an additional 10.0 µg/L of the standard solution were also prepared by following the same procedure as those of the plant preparation. The spiked sample provides a percent recovery estimate for each sample matrix.

Optimized homogenization and extraction protocol

The preliminary analysis method was further modified[6] to reduce matrix effects by adding a hexane partitioning step (hexane clean-up step). The geranium samples remained frozen at −20 °C prior to analysis and thawed for sample preparation. The wet geranium leaves and stems were cut into 1 to 2-cm pieces and subsequently ground with a high-speed grinder into finely chopped pieces. Then, 10 g of the chopped sample were weighed and placed into a standard food blender with 80 mL of 0.5 N HCl and homogenized at the highest blend setting for two minutes. The blended mixture was transferred into a 100-mL volumetric flask, and the blade and blender cup were rinsed with 15 mL of 0.5 N HCl and poured into the 100-mL volumetric flask. The blended geranium mixture was extracted by sonication for one hour at 50 °C. This solution was centrifuged at 3700 × g for ten minutes after cooling and filling to volume with 0.5 N HCl. Four mL of the supernatant and 2 mL of hexane were added to a 15-mL glass centrifuge tube with screw cap. This mixture was shaken by a vortex mixer for thirty seconds. The mixture was then centrifuged at 2000 × g for five minutes. The aqueous layer was filtered and analyzed by LC-MS/MS. For all sample analyses, a blank was analyzed with each sample to verify no carryover occurred from the previous analysis. For standard addition analysis, spiked samples were prepared by spiking standard prior to the blending process, such that the final added concentration was 15.0 and 25.0 µg/L in the volumetric flask.

This optimized method added and modified existing steps (grinding, sonication, and centrifuging) to the original extraction protocol to maximize the extraction efficiency of 1,3-DMAA and 1,4-DMAA from the plant matrix. The reduction of plant material extracted and increased volume of extractant resulted in a more practical extraction procedure and minimized sample handling errors. The sonication temperature was increased to 50 °C to increase the breakup and dissolution of the plant material in the acid extract and increase solvation of the analytes. The additional hexane extraction step minimized concentrations of the non-polar plant material in the 0.5 N HCl extraction solution. The non-polar plant material likely caused matrix effects during analysis

by causing ion suppression in the ESI source. The combination of these steps provides an extract that contains a more representative concentration of 1,3-DMAA and 1,4-DMAA and a reduction of matrix effects. This means that the performance of the extraction method improves and this is demonstrated by the large improvement in percent recovery.

HPLC-MS/MS instrumentation

The LC-MS/MS system consists of an Agilent 1100 HPLC system equipped with an autosampler, coupled to a triple quadrupole mass spectrophotometer (Waters Quattro Ultima) operated in ESI+ mode. The injection volume was 100 µL with separation performed on a Phenomenex Kinetex C18 phase column (4.6 × 150 mm, 2.6 µm) with a column temperature set at 25 °C and flow rate at 0.4 mL/min. The HPLC eluent ratio was 82:18 of mobile phase A (1% of formic acid in reagent water) to mobile phase B (acetonitrile). The column effluent was split at a ratio of 1:1 prior to introduction to the mass spectrometer.

The mass spectrometer operating conditions were as follows: the capillary voltage was 3.0 kV, the cone voltage was 20 V, the source temperature was set at 120 °C with a flow of 108 L/hr, and the desolvation temperature was 350 °C with a flow of 635 L/hr. The dwell time was 0.5 second and the interscan delay was 0.1 second. The collision voltage was set to 8 eV with a collision gas (argon) pressure at 7 psi. The detection of the analytes was done using the MRM function with a pair of mass transitions of 116/99.7 m/z and 116/57 m/z to produce a single chromatogram for both 1,3-DMAA and 1,4-DMAA.

All chromatogram integrations were performed with Waters MassLynx MS software. Each chromatogram was prefiltered with a peak-to-peak noise amplitude of 2000. Chromatograms were submitted to a Savitzky Golay[10] smoothing method within the MassLynx software. The Savitzky Golay method takes an average of the intensities of the data points weighted by a quadratic curve.

The LC-MS/MS total analysis time was 10 minutes. Figure 2 presents a typical standard chromatogram of a 20 µg/L standard of 1,3-DMAA and 1,4-DMAA. Additional standards are presented in the supplementary materials (Figs. S1–S3). It is important to mention that the compound 1,3-DMAA has two chiral centers that result in four stereoisomers (Fig. 1). These stereoisomers include two diastereomers that have different physical properties and can be separated. Therefore, 1,3-DMAA is detected as two peaks in the chromatogram. All values referenced to 1,3-DMAA_total or 1,3-DMAA are calculations based on the summation of both peak areas.[2,6,9] The compound 1,4-DMAA exists as two enantiomers which cannot be separated. Therefore, only one peak was detected for 1,4-DMAA.

Results and Discussion

Detection limits, accuracy, precision, and linearity studies

Before sample analysis was conducted, detection limit,[11–13] accuracy,[14] precision,[14] and linearity[8] studies were conducted to evaluate and ensure acceptable instrument performance. The typical practice for United States Environmental Protection Agency (USEPA) MDL studies in the laboratory is to construct a 5-point calibration curve and analyze a check standard halfway between the two lowest calibration points. The USEPA MDL reported here represents the

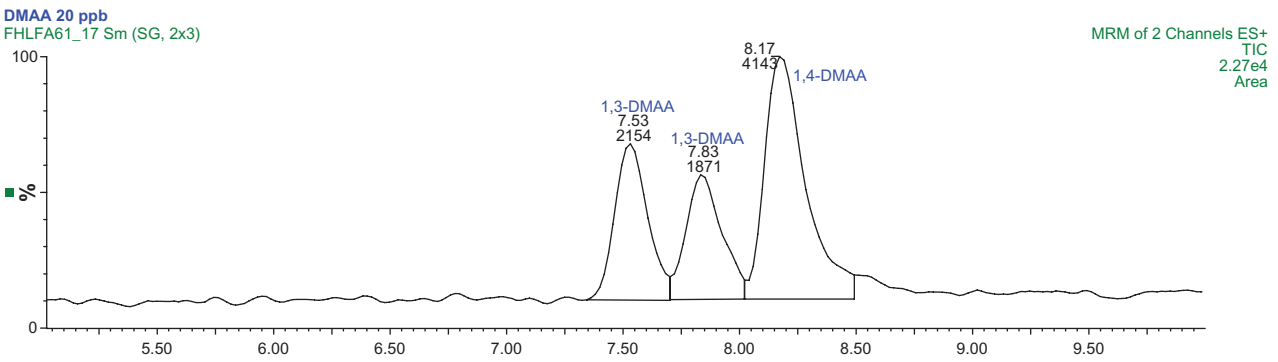

Figure 2. Typical MRM Chromatogram at 20 µg/L each for 1,3 and 1,4-DMAA analytes.
Note: The retention times for the 1,3-DMAA diastereomers are 7.53 and 7.83 minutes, and 1,4-DMAA retention time is 8.17 minutes.

lowest concentration distinguishable from noise and determined on the variation of the analytical signal of a check standard expected to be within a factor of 2 to 5 of the detection limit. At these analytical conditions, the MDL study provides a worst-case estimate of the analyzer performance. The accuracy of the analysis is estimated using the mean percent recovery of the check standard analysis.[14] The precision is estimated as the percent relative standard deviation (% RSD).[14]

Another estimate for the detection limit is the propagation of uncertainty MDL (Unc. MDL).[13] The Unc. MDL is determined using the standard deviations of the slope (m), y-intercept (b), and signal (y) as determined by the LINEST function in Microsoft Excel. These standard deviations are then used to propagate and determine the error on "x" in the linear regression line.[13] The propagated error represents the lowest concentration of analytical significance.

Detailed MDL, accuracy, and precision studies of 1,3-DMAA and 1,4-DMAA are presented in Tables 1 and 2, respectively, for all sample analysis conducted (Analysis Sets 1 to 3). The reported values for Analysis set 1 were based on the preliminary extraction protocol. Analysis Sets 2 and 3 were conducted using a hexane clean-up step as well as standard addition analysis. Typically, an MDL, accuracy and precision study was conducted with two different check standard concentrations prior to each set of sample analysis. For Analysis Sets 1 and 2, the MDLs at 3.0 µg/L were based on the calibration curves from 1 to 20 µg/L (low range calibration). The MDLs at 8.0 µg/L were based on the 3 to 100 µg/L calibration curves (high range calibration). In Analysis Set 3, the calibration curve for the 2.0 µg/L check standard was 1 to 100 µg/L, and the calibration curve for the 3.0 µg/L check standard was 2 to 100 µg/L. The R^2 values for all studies with both DMAA species were greater than 0.99.

The MDL values[11,12] for 1,3-DMAA range from 0.6 to 3.2 µg/L and for 1,4-DMAA, range from 0.8 to 2.7 µg/L. Accuracy[14] for 1,3-DMAA ranges between 60% and 126% and for 1,4-DMAA, ranges between 48% and 127%. The precision (estimated as % RSD)[14] for 1,3-DMAA is in the range of 9% to 35%, and for 1,4-DMAA, precision ranges between 10% and 30%. With the exception of one mean percent recovery analysis in Analysis Set 2, the reported mean percent recoveries and % RSD are within the guidelines set by the USEPA[14] for check standard analysis. The USEPA reports that mean percent recovery can range from 50% to 150%, and the % RSD can be up to 30% when samples are analyzed within a factor of 2 to 5 of the MDL.[14] As the MDL factor decreases, the % RSD of the check standard analysis increases, and below an MDL factor of 2, the % RSD can dramatically increase beyond 30%.[15]

Ideally, MDL, accuracy and precision studies should provide estimates that are similar to each other.[15–17] Further confidence of these MDL values is gained when the USEPA MDLs are compared to the Unc. MDL. Both sets of detection limit values are within 2 µg/L of each other in absolute terms and within a factor of 5 in all cases. This similarity indicates the MDL values for the calibration and analysis protocols are realistic estimates for both 1,3-DMAA and 1,4-DMAA.

A linearity study was conducted to estimate the upper limit of linearity for the LC-MS/MS analysis.[8] A calibration curve was prepared and analyzed over the range of 1 to 250 µg/L for 1,3-DMAA and 1,4-DMAA, with both species being linear over the entire range as evidenced by the excellent R^2 values (>0.99). The linearity study resulted in a linear regression

Table 1. Detection limits, accuracy, and precision studies for 1,3-DMAA for all sample analysis.

Analysis Set	Check standard (µg/L)	USEPA MDL (µg/L)	Unc. MDL (µg/L)	Mean % recovery	% RSD	MDL factor	Equation of linear regression	r^2
Analysis Set 1	3.0	1.1	0.4	126	9	2.8	y = 195.81x – 19.049	0.999
	8.0	1.8	3.4	73	10	4.5	y = 148.84x + 420.8	0.999
Analysis Set 2	3.0	2.3	0.5	71	35	1.3	y = 121.94x + 72.43	0.998
	3.0	1.8	0.8	95	20	1.7	y = 90.587x – 43.177	0.994
	8.0	2.5	1.5	62	16	3.2	y = 114.66x + 199.46	0.999
	8.0	3.2	1.4	60	21	2.5	y = 78.45x + 131.18	0.999
Analysis Set 3	2.0	1.4	2.6	103	21	1.5	y = 111.83x + 60.259	0.996
	3.0	0.6	1.4	63	10	4.9	y = 135.07x + 190.33	0.999

Table 2. Detection limits, accuracy, and precision studies for 1,4-DMAA for all sample analyses.

Analysis Set	Check standard (µg/L)	USEPA MDL (µg/L)	Unc. MDL (µg/L)	Mean % recovery	% RSD	MDL factor	Equation of linear regression	r^2
Analysis Set 1	3.0	1.4	0.7	127	12	2.1	y = 201.78x – 78.268	0.996
	8.0	2.7	4.6	60	18	2.9	y = 147.57x + 552.07	0.998
Analysis Set 2	3.0	2.0	0.6	73	30	1.5	y = 130.39x + 31.787	0.996
	3.0	0.9	0.6	93	10	3.4	y = 85.06x – 38.131	0.997
	8.0	2.4	2.9	48	20	3.3	y = 109.18x + 340.17	0.995
	8.0	2.1	0.9	81	10	3.9	y = 79.501x – 7.9734	0.999
Analysis Set 3	2.0	0.8	2.6	98	13	2.4	y = 95.314x + 76.382	0.996
	3.0	0.8	1.6	76	11	3.7	y = 121.63x + 118.7	0.999

equation for 1,3-DMAA of y = 149.08x + 380.91 and for 1,4-DMAA of y = 148.05x + 473.94.

DMAA concentrations in the plant material

The reported concentration of the DMAA species in the geranium herb was determined using the calculated concentration from the calibration curve, final extraction volume, and mass of geranium (Equation 1, below). The MDL, accuracy, and precision studies (Tables 1 and 2) were conducted with prepared standards in solution (no extraction). However, the MDLs in the analyzed plant would vary with the amount of plant mass used and the final extracted volume. For Analysis Set 1, the amount of plant material used was 50 g extracted into 25.00 mL. This resulted in MDLs that ranged from 0.6 to 1.4 ng DMAA/g geranium. In Analysis Sets 2 and 3, 10 g of plant material were extracted into 100.00 mL, which resulted in MDLs ranging from 6 to 32 ng DMAA/g geranium. While the MDLs increased for the second extraction method, the percent recovery of DMAA analysis also increased for all samples. The increase in percent recovery is likely due to the hexane clean-up step as well as a more practical increase in the extraction solvent volume. If the mass of plant material were doubled, the MDLs of the optimized extraction protocol would likely increase by a factor of two.

$$DMAA_{geranium}(ng/g) = [[DMAA_{cal.curve}\ (\mu g/L) \times \text{Extraction volume (L)}] \div \text{Geranium mass (g)}] \times 1000 \quad (1)$$

Authenticated *Pelargonium graveolens* samples

The *Pelargonium graveolens* (geranium) samples were collected and authenticated as all belonging to the genus and species *Pelargonium graveolens* by Xu YouKai of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Samples were collected from three regions in China: Changzhou, Guiyang, and Kunming, during three different harvest seasons. The Chinese Academy received the geranium herbs as potted plants originally grown in the field. Multiple plants (ranging from two to ten in number) were collected from each location. The plants from each location were combined prior to shipment to The University of Memphis. Therefore, concentrations of 1,3-DMAA and 1,4-DMAA in individual plants and variations thereof are not reported here. The samples were sent by express airmail from Dr. Yi Jin of Yunnan University directly to the University of Memphis where the samples were immediately stored at –20 °C. Analysis Sets 1 and 2 consisted of a Changzhou sample collected on June 9, 2011 (Changzhou S11-1 and Changzhou S11-2), a Kunming, China, sample collected March 20, 2012 (Kunming 1 and 2); a Guiyang, China, sample collected March 16, 2012 (Guiyang 1 and 2); and an additional Changzhou, China, sample collected on March 10, 2012 (Changzhou 1). Analysis Set 3 consisted of a Changzhou sample collected on May 18, 2012 (Changzhou 3), a Guiyang sample collected May 20, 2012 (Guiyang 3), and a Kunming sample collected May 23, 2012 (Kunming 3). The Changzhou S11 sample was received from Intertek Labs (Detroit, MI, USA) and frozen upon arrival. The Changzhou S11 sample is an identical sample previously analyzed and reported by Li,[6] providing an inter-laboratory analysis of a sample. The numbers for each region identifier signify the various Analysis Sets.

Sample Analysis set 1: preliminary extraction protocol

The concentrations of 1,3-DMAA and 1,4-DMAA in the three winter geranium samples and Changzhou S11 sample are presented in Table 3. The Changzhou S11-1 analysis was conducted in duplicate and the winter samples were analyzed in singlet. A spike sample was analyzed to determine the percent recovery for that particular plant sample. There is no reported spike analysis for Changzhou 1 due to a sample loss during analysis. No additional sample was available. The percent recovery of the spike was calculated using equation 2:[13]

$$\text{Percent Recovery} = [[\text{Spike conc. } (\mu gL) - \text{Unspiked conc. } (\mu gL)] \div 10(\mu gL)] \times 100\% \quad (2)$$

Of the four samples in Analysis Set 1, only the Changzhou S11-1 and Changzhou 1 sample contained 1,3-DMAA and 1,4-DMAA above the MDLs of the method (Table 3). Figures 3 and 4 present an MRM chromatogram of Changzhou S11-1 and Changzhou 1 samples, respectively. Additional sample and spike chromatograms are presented in the supplementary materials (Figs. S4–S8). The average concentration of 1,3-DMAA in the Changzhou S11-1 sample was 94.7 ± 15.1 ng/g geranium, with a percent recovery of 19% on the 10 µg/L spike. The average concentration of 1,4-DMAA in Changzhou S11-1 was 13.5 ± 1.8 µg/L with a 65% recovery on a 10 µg/L spike. The concentrations of 1,3-DMAA and 1,4-DMAA in Changzhou 1 samples were 213 and 52 ng/g respectively. The reported 1,3-DMAA concentrations for Changzhou S11-1 and Changzhou 1 samples were outside the calibration range but within the linearity of the analyzer. A 1:1 dilution of both samples was analyzed and resulted in calculated concentrations within 9% of the original concentration reported in Table 3.

While the percent recovery of the DMAA species is not ideal, the relative concentrations should be considered for the spike. For Changzhou S11-1 sample, the concentrations of 1,3-DMAA in volumetric flask after extraction averaged 190 µg/L. The % RSD error of analysis from the MDL study was of 9% to 10% for Analysis Set 1 and translates to ~18 µg/L error. This is more than twice the 10 µg/L spike and thus a likely contributor to the low percent recovery (high error). When 1,4-DMAA was examined, the 10 µg/L spike addition was outside the error of analysis (2.7 µg/L) and gave a more reasonable 65% recovery. Additionally, the low percent recoveries across all samples indicated the presence of a matrix effect. Previous reports[6] have suggested that extraction protocols are likely to be extracting lipids from the cell membranes and contributing to ion suppression in the ESI source.

Analysis Set 2: optimized extraction protocol analysis of Changzhou S11 and winter geranium samples

The matrix effect identified in Analysis Set 1 was minimized by the addition of a hexane clean-up step. Additionally, the optimized method was more efficient as it used less plant sample mass per extraction. This efficiency provided an opportunity to re-analyze Changzhou S11, Kunming, and Guiyang winter samples. Each sample was extracted and analyzed with two different spike concentrations (15.0 µg/L and 25.0 µg/L) for both 1,3-DMAA and 1,4-DMAA and in duplicate. The spiked samples were analyzed concurrently with the unspiked ones, and the percent

Table 3. Analysis Set 1: preliminary extraction protocol results of geranium samples from Changzhou, Kunming, and Guiyang.

	1,3-DMAA			1,4-DMAA		
	Sample (ng/g)	Spike level (µg/L)	Percent recovery (%)	Sample (ng/g)	Spike level (µg/L)	Percent recovery (%)
Changzhou S11-1	94.7 ± 15.1	10.0	19	13.5 ± 1.8	10.0	65
Kunming 1	<0.5*	10.0	44	<0.7*	10.0	32
GuiYang 1	<0.5*	10.0	36	<0.7*	10.0	23
Changzhou 1	213	N/A	N/A	52.0	N/A	N/A

Note: *The results are less than the MDL values.

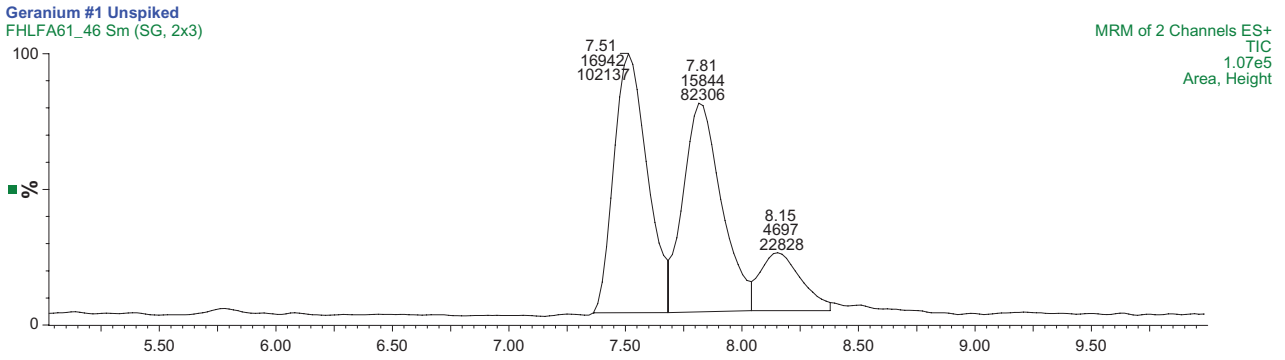

Figure 3. A MRM chromatogram of Changzhou S11-1 sample.
Notes: The first two peaks are 1,3-DMAA diastereomer pairs with retention times of 7.51 minutes and 7.81 minutes. The 1,4-DMAA peak retention time is 8.15 minutes. The chromatogram is produced using two mass transitions 116/99.7 m/z and 116/57 m/z.

recovery was subsequently calculated.[13] Detailed analysis results are presented in Table 4.

Changzhou S11 concentrations were expected to be high and thus analyzed on the high range calibration of 3 to 100 µg/L of both DMAA species. The concentrations of 1,3-DMAA and 1,4-DMAA were 254 ng/g and 39.8 ng/g, respectively, and an optimized extraction chromatogram of Changzhou S11-2 is presented in Figure 5. The percent recovery[13] for 1,3-DMAA was approximately 55% for both spike levels. Both Kunming and Guiyang (Fig. 6) samples were analyzed using the low range calibration curves (1 to 20 µg/L of each DMAA species). The concentrations of 1,3-DMAA and 1,4-DMAA are reported in Table 4. All are less than the MDL of the analysis. The percent recovery for all remaining samples ranged from 63% to 107%, indicating that the matrix effect previously identified was substantially mitigated by the optimized extraction protocol. The chromatograms for samples and 15.0 µg/L spikes of Analysis Set 2 are also presented in the supplementary materials (Figs. S9–S14).

A comparison of the two extraction protocols using Changzhou S11 geranium sample demonstrates that the preliminary extraction protocol underestimated the concentrations of both DMAA species as indicated by the percent recovery results. However, it is clear that Changzhou S11 geranium samples contain 1,3-DMAA species and the concentrations are well above the MDL of both analysis. In contrast, Kunming and Guiyang samples did not contain 1,3-DMAA or 1,4-DMAA species at significant concentrations above the MDL of analysis (20 ng/g).

Analysis set 3: optimized extraction protocol of summer geranium samples

An additional round of samples was collected from a summer harvest of geranium plants and analyzed using the same protocols from Analysis Set 2 (with two spike levels, in duplicate). The Changzhou 3 sample (Fig. 7)

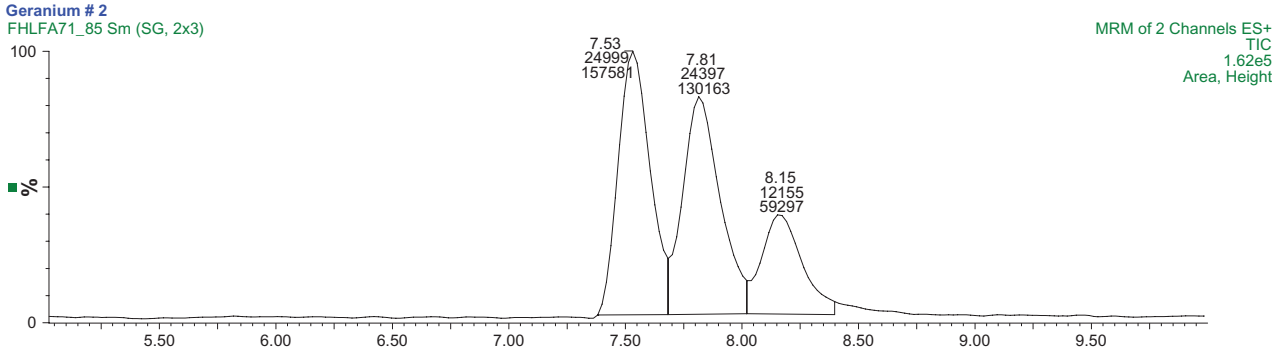

Figure 4. A MRM chromatogram of the Changzhou 1 sample.
Notes: The first two peaks are 1,3-DMAA diastereomer pairs with retention times of 7.51 minutes and 7.81 minutes. The 1,4-DMAA peak retention time is 8.15 minutes. The mass transitions used are 116/99.7 m/z and 116/57 m/z.

Table 4. Analysis set 2: optimized extraction protocol results of geranium samples from Changzhou S11, Kunming, and Guiyang.

	1,3-DMAA			1,4-DMAA		
	Sample (ng/g)	Spike level (µg/L)	Percent recovery (%)	Sample (ng/g)	Spike level (µg/L)	Percent recovery (%)
Changzhou S11-2	254 ± 17	15.0	54 ± 5	39.8**	15.0	76 ± 2
		25.0	55 ± 8		25.0	65 ± 1
Kunming 2	<20 ± 4*	15.0	83 ± 11	<14 ± 8	15.0	78 ± 10
		25.0	67 ± 1		25.0	63 ± 5
Guiyang 2	<20 ± 4*	15.0	107 ± 23	<14 ± 8	15.0	82 ± 16
		25.0	81 ± 2		25.0	78 ± 6

Notes: *The results are less than the MDL values; **one duplicate was less than MDL for the sample (23.9 ng/g).

contained 1,3-DMAA and 1,4-DMAA concentrations of 68.8 ± 36.5 ng/g and 118 ± 45 ng/g, respectively (Table 5). Both Kunming 3 and Guiyang 3 had concentrations of 1,3-DMAA and 1,4-DMAA below the MDL (less than 10 ng/g). These results are consistent with the previous winter sample analysis. Both DMAA species were detected and quantified in the Changzhou samples, but no DMAA species were detected above the MDL in Kunming and Guiyang samples (See Supplementary materials Figs. S15–S20). The percent recovery for all samples was excellent and ranged between 64% and 86%.

Winter versus summer sample analysis

Previous research has shown that concentrations of chemical species in natural products can be highly variable.[18,19] A seasonal comparison is possible between the winter harvest (March 2012) and the summer harvest (May 2012) for Kunming, Guiyang and Changzhou samples. Neither the winter nor summer harvest samples of Kunming and Guiyang samples contained 1,3-DMAA or 1,4-DMAA species above the MDLs of the analysis. However, Changzhou sample resulted in similar concentrations of 1,3-DMAA and 1,4-DMAA in the June 2011 and March 2012 samples. From March 2012 to May 2012, 1,3-DMAA resulted in a factor of 3 decrease in concentration while 1,4-DMAA about doubled in concentration. These results indicate a potential seasonal effect of 1,3-DMAA and 1,4-DMAA concentrations in agreement with previously reported research discussing environmental effects on chemical composition.[18,19] It is also possible the concentrations of 1,3-DMAA in Changzhou winter samples were higher due to an apparent underestimation of 1,3-DMAA concentrations by the preliminary extraction protocol as evidenced by Changzhou S11 analysis.

Standard addition analysis of 1,3-DMAA and 1,4-DMAA

A standard addition analysis protocol was developed for sample analysis. Standard addition analysis

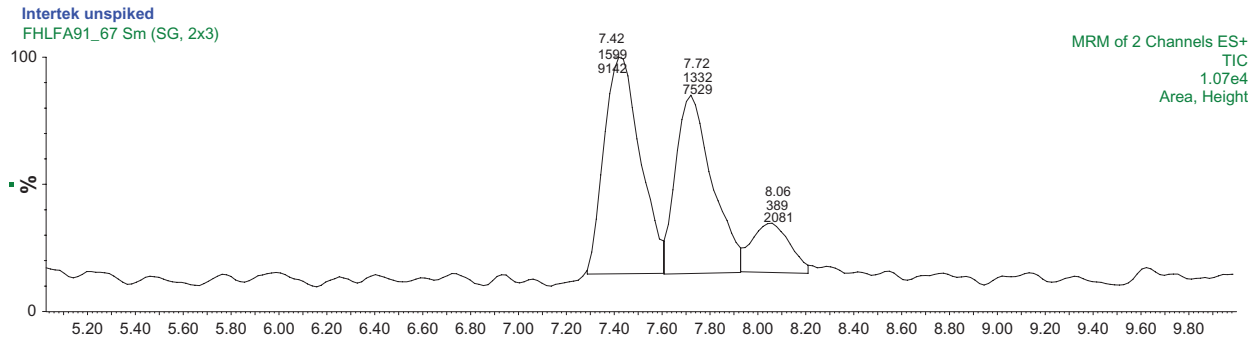

Figure 5. A MRM chromatogram of the optimized extraction protocol for Changzhou S11-2 showing the presence of 1,3-DMAA diastereomers (peaks 1 and 2) and 1,4-DMAA (peak 3).
Note: The mass transitions used are 116/99.7 m/z and 116/57 m/z.

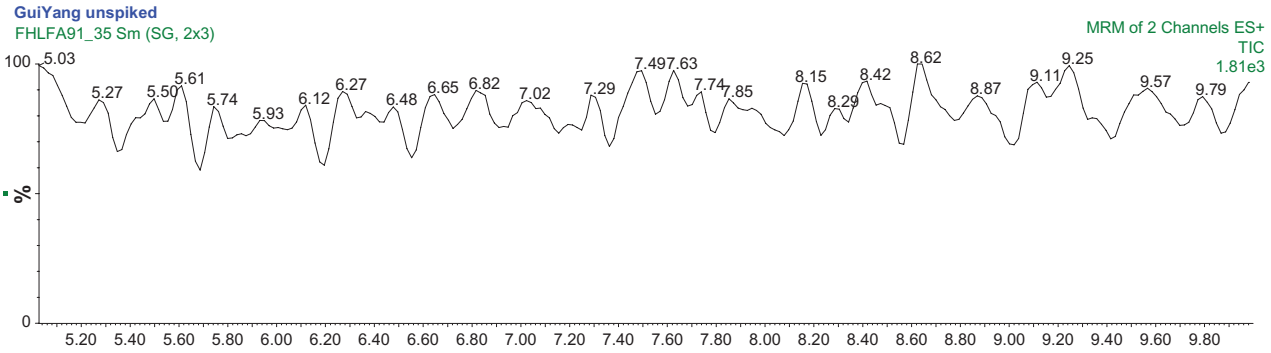

Figure 6. A typical MRM chromatogram of the Guiyang 2 sample demonstrating the absence of 1,3-DMAA and 1,4-DMAA in the geranium plant.
Note: The mass transitions used are 116/99.7 m/z and 116/57 m/z.

compensates for matrix effects found in geranium plants caused by chemical species other than DMAA affecting analytical signal (either positive or negative).[13] In the standard addition method, known quantities of the 1,3-DMAA and 1,4-DMAA standards are added to the sample extract. This is termed "spiking" the sample. The added standard is affected by matrix effects just as the analyte in the sample. The unknown concentration can then be derived from a plot of signal versus spike concentration as long as the analyte has been previously established to have a linear signal response. Thus, the standard addition method resolves matrix interferences present in the complex geranium sample composition.[13]

The standard addition protocol was applied to both Analysis Sets 2 and 3 (Changzhou S11, Kunming, and Guiyang winter samples and Changzhou, Kunming, and Guiyang summer samples). For this study, a three-point standard addition plot was constructed using the unspiked sample, a 15.0 µg/L spike each of 1,3-DMAA and 1,4-DMAA, and a 25.0 µg/L spike each of 1,3-DMAA and 1,4-DMAA. The signal was plotted against the spike concentration (0, 15, and 25 µg/L), and a linear regression analysis was performed. The slope (m) and y-intercept (b) of the calibration curve were used to calculate the concentration of analyte (x) in the sample.[13] The equation for determining the x-intercept is $x = -b/m$, and in standard addition, the negative of the x-intercept is the concentration present in the unspiked sample.

The standard addition analysis results showed some matrix effects were still present in the optimized procedure and the external calibration analysis likely underestimated DMAA concentrations. However, the standard addition analysis agreed overall with the external calibration results. Samples reported to contain 1,3-DMAA by external calibration also contained 1,3-DMAA by standard addition. Concentrations of 1,3-DMAA species were quantified in both Changzhou S11-2 and Changzhou 3 samples at 496 ± 46 ng/g and 97 ± 20 ng/g, respectively. The concentrations of 1,4-DMAA in Changzhou S11-2 and Changzhou 3 samples were 68 ± 7 ng/g and 162 ± 48 ng/g, respectively. All concentrations were

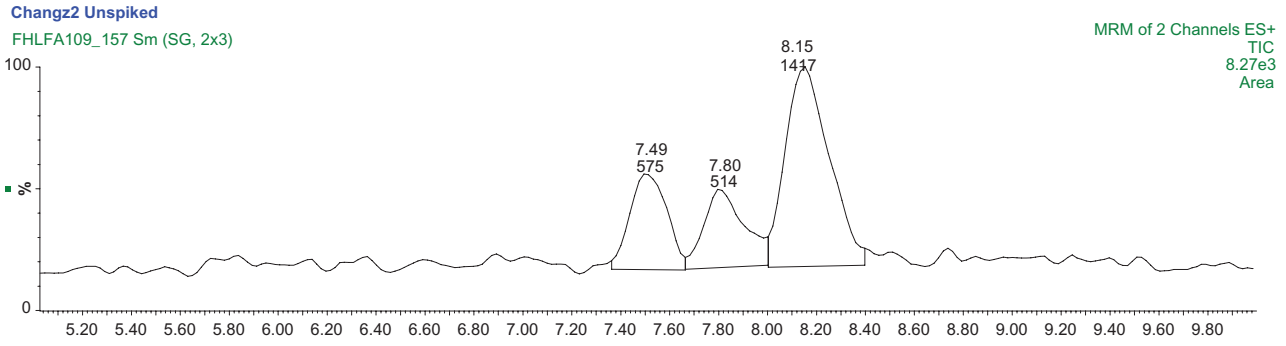

Figure 7. A MRM chromatogram of Changzhou 3 sample showing the presence of 1,3-DMAA at a lower concentration than 1,4-DMAA.
Note: Mass transitions are 116/99.7 m/z and 116/57 m/z.

Table 5. Analysis set 3: optimized extraction protocol results of geranium summer samples from Kunming, Guiyang, and Changzhou.

	1,3-DMAA			1,4-DMAA		
	Sample (ng/g)	**Spike level (µg/L)**	**Percent recovery (%)**	**Sample (ng/g)**	**Spike level (µg/L)**	**Percent recovery (%)**
Kunming 3	<10 ± 6*	15.0	68 ± 3	<8.2 ± 0.3*	15.0	64 ± 2
		25.0	74 ± 6		25.0	75 ± 9
Guiyang 3	<10 ± 6*	15.0	75 ± 4	<8.1 ± 0.2*	15.0	78 ± 1
		25.0	81 ± 8		25.0	84 ± 6
Changzhou 3	68.8 ± 36.5	15.0	76 ± 13	118 ± 45	15.0	86 ± 4
		25.0	79 ± 13		25.0	77 ± 7

Note: *The results are less than the MDL values.

well above the MDL of the analysis and clearly demonstrated 1,3-DMAA and 1,4-DMAA were present in geranium herbs from the Changzhou region.

The standard addition results for winter and summer samples of Kunming and Guiyang agreed with the external calibration results. Concentrations of 1,3-DMAA and 1,4-DMAA were all less than the MDL previously reported, or so close to the MDL that the confidence of analysis was extremely low. One of the Kunming 3 duplicates resulted in a 1,3-DMAA concentration of 21 ng/g, while the other duplicate was below the MDL of 14 ng/g. Similarly, one of the Kunming 2 duplicates resulted in a 1,4-DMAA concentration of 10 ng/g, whereas the other duplicate had concentrations less than the 20 ng/g MDL of that particular analysis.

Measurement of the diastereomer ratios of 1,3-DMAA in the Changzhou geranium samples

Zhang et al[5] measured the diastereomer ratios (reported as first peak/second peak) of synthetic standards and dietary supplements containing 1,3-DMAA using GC-FID analysis. The reported results showed the diastereomer ratio of a Sigma-Aldrich standard of 1,3-DMAA was 1.22 ± 0.06 and the ChromaDex standard ratio was 1.42 ± 0.09. The dietary supplements had identical ratios to those of the standards suggesting that both standards and supplements were of synthetic origin.

In this report, both pairs of diastereomers were detected in the Changzhou region samples as well as the synthetic calibration standards. By inspection of the chromatograms (Figs. 3, 4, 5, and 7), both standards and geranium samples present similar diastereomer ratios. Quantitatively, the average ratio of 1,3-DMAA diastereomers (first peak/second peak) in typical 20, 50 and 100 µg/L calibration standards is 1.14 ± 0.08. The diastereomer ratio of Changzhou S11-1 sample was 1.10 ± 0.01, Changzhou 1 was 1.02, Changzhou S11-2 was 1.25 ± 0.03, and Changzhou 3 was 1.16 ± 0.10. The results of the geranium plant diastereomer ratios are similar to the ratios of the synthetic standards presented here, as well as the standards and supplements analyzed by Zhang et al. This indicates that supplements containing both 1,3-DMAA diastereomer pairs could be naturally produced and extracted from geranium plants.

Conclusion

In conclusion, geranium plants (*Pelargonium graveolens*) from three different regions of China (Kunming, Guiyang, and Changzhou) and three different harvests (June 2011, March 2012, and May 2012) were analyzed for 1,3-DMAA and 1,4-DMAA. An extraction and HPLC-MS/MS analysis method was used to determine concentrations of 1,3-DMAA and 1,4-DMAA with both external calibration and standard addition analysis. The extraction and external calibration analysis likely suffered from matrix effects and thus underestimated concentrations of 1,3-DMAA and 1,4-DMAA in geranium plants. The matrix effects were largely solved by the standard addition analysis, as expected. This demonstrates that future analysis should use standard addition to minimize matrix effects and increase confidence of analysis with little additional labor. All extraction and calibration protocols reported 1,3-DMAA and 1,4-DMAA concentrations in geranium plants from the Changzhou region

of China above the reported MDLs. The reported concentrations of 1,3-DMAA ranged from 68 to 496 ng/g and 1,4-DMAA ranged from 13 to 162 ng/g. Similarly, 1,3-DMAA and 1,4-DMAA were not detected above the MDL in samples from Guiyang and Kunming regions. To the best of the authors' knowledge, this is the first reported inter-laboratory analysis confirming the presence of 1,3-DMAA in a geranium plant (specifically Changzhou S11 sample). Finally, the diastereomer ratios of the 1,3-DMAA in geranium plants from Changzhou are similar to those of the synthetic standards. This indicates that 1,3-DMAA could be a natural product extract, fulfilling a requirement of the Dietary Supplement Health and Education Act.[20]

The results reported here provide evidence that 1,3-DMAA naturally occurs in geranium plants in agreement with Li et al,[6] but clearly in disagreement with other previously reported articles by well-respected chemists and organizations.[4,5] However, this may not be a case of right or wrong. In analytical chemistry, the critical review of data is important for explaining differences in reported results. These differences can also provide insight into why analysis of seemingly identical plant species can result in very different outcomes. Khan has published an extensive review showing that it is not uncommon for plants of different locations to exhibit variations in their chemical compositions.[18] For example, studies show that fluctuating geographical dynamics such as water stress and nutrient availability in the soil are associated with the variations in cyanide concentration in the cassava plant.[19]

The published research to date includes a substantial amount of geranium plant and oil analysis.[1–6] However, until now, none of the samples analyzed have been identical or reported as from the same region. Thus, regional environmental variations[18,19] could explain the presence of 1,3-DMAA in the Changzhou S11, Changzhou March 2012, and Changzhou May 2012 samples and the absence of 1,3-DMAA concentrations in Kunming and Guiyang geranium samples reported here; the Indian and Mississippi samples reported by ElSohly et al,[4] the France, Egypt, and New Zealand samples reported by Lisi et al,[3] and the China and Egypt samples reported by Zhang et al.[5] A possible solution to this discrepancy would be a multiple laboratory and blind analysis of identical samples expected to have 1,3-DMAA (such as Changzhou region samples) as well as samples that are not expected to contain 1,3-DMAA. Using this approach, a satisfactory answer for the national regulatory agencies as well as the commercial interests could be provided.

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Author Contributions
Conceived and designed the experiments: PSS, HLF, PLR. Analysed the data: HLF, PLR, PSS. Wrote the first draft of the manuscript: HLF, PLR, PSS. Contributed to the writing of the manuscript: HLF, PLR, PSS. Agree with manuscript results and conclusions: HLF, PLR, PSS. Jointly developed the structure and arguments for the paper: HLF, PLR, PSS. Made critical revisions and approved final version: HLF, PLR, PSS. All authors reviewed and approved of the final manuscript.

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PSS has received consulting fees from CirQuest Labs for consulting work on analysis for pharmaceutical and implantable devices. All other authors disclose no competing interests.

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Supplemental Materials

Chromatograms of typical blanks, standards, and sample spikes

Figure S1 is an example of a typical, original (bottom) and smoothed (top) chromatogram for a sample blank. Figures S2 and S3 are examples of 1,3 and 1,4-DMAA standard chromatograms. Two concentrations are shown: 3 (Fig. S2) and 20 (Fig. S3) µg/L each DMAA. 1,3-DMAA elutes as the first two peaks, followed by 1,4-DMAA as the third peak. Figures S4 to S20 are chromatograms for each geranium herb sample. Unspiked and spiked chromatograms are shown for each sample where possible. Each chromatogram is labeled by the corresponding table number found in the paper. As with the standards, 1,3-DMAA elutes as the first two peaks followed by 1,4-DMAA as the third peak. Figures S4 to S8 are typical chromatograms from Analysis set 1 and were used to determine the concentration of Guiyang 1, Kunming 1, and Changzhou S11-2 in Table 3. Figures S9 toS14 are typical chromatograms from Analysis set 2 and were used to determine the concentration of Guiyang 2, Kunming 2, and Changzhou S11-2 in Table 4. Figures S15 to S20 are typical chromatograms from Analysis set 3 and were used to determine the concentration of Guiyang 3, Kunming 3, and Changzhou 3 in Table 5.

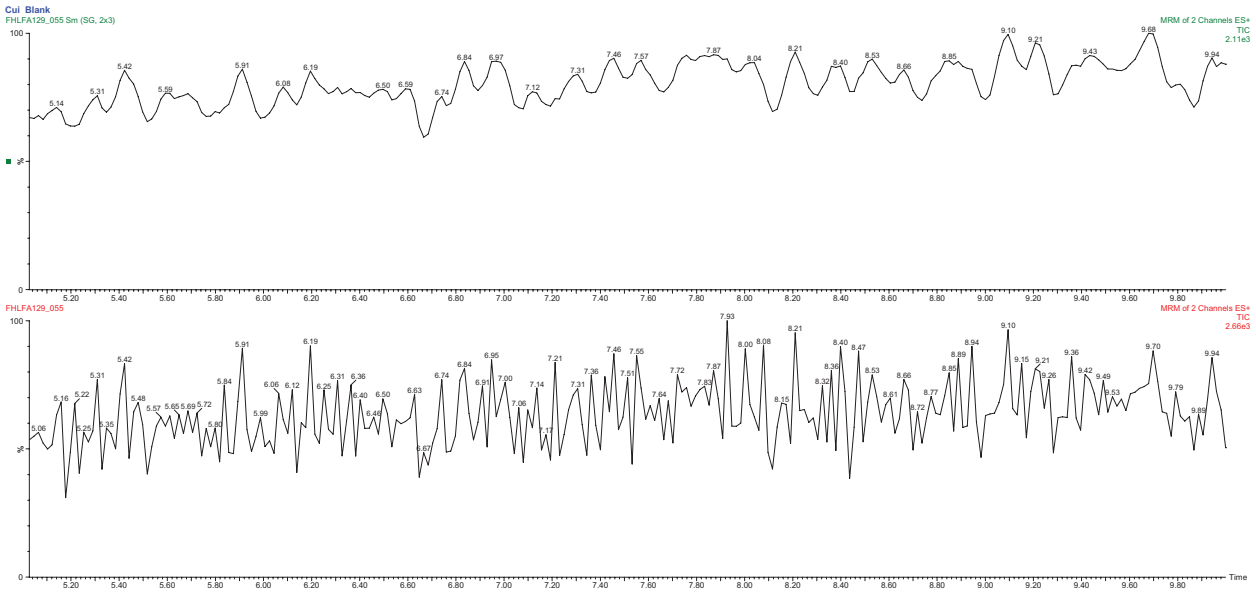


Figure S1. An example of a blank chromatogram.
Notes: The blank sample is prepared in the same way as an unspiked sample but there is no addition of geranium herb to the blender. The original chromatogram is presented on the bottom and the smoothed chromatogram is presented on the top.

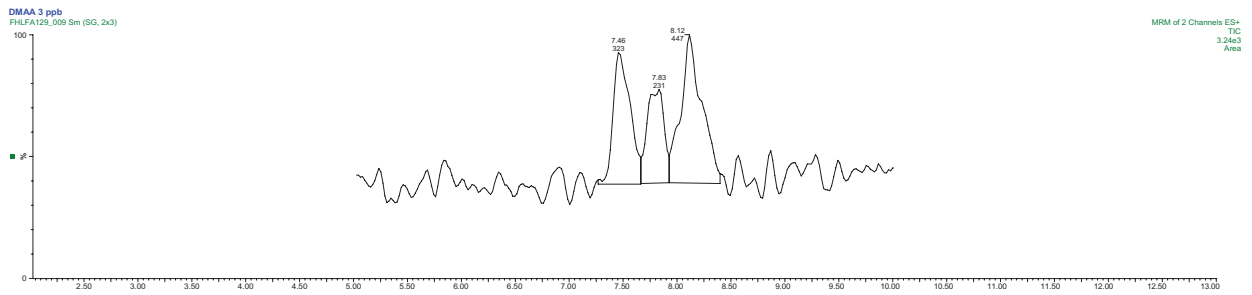


Figure S2. Typical MRM chromatogram of 3 µg/L 1,3-DMAA and 1,4-DMAA.

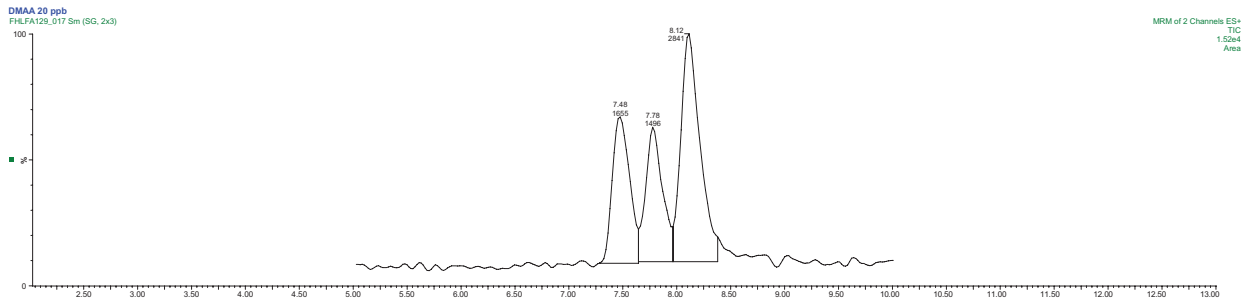


Figure S3. Typical MRM chromatogram of 20 μg/L 1,3-DMAA and 1,4-DMAA.

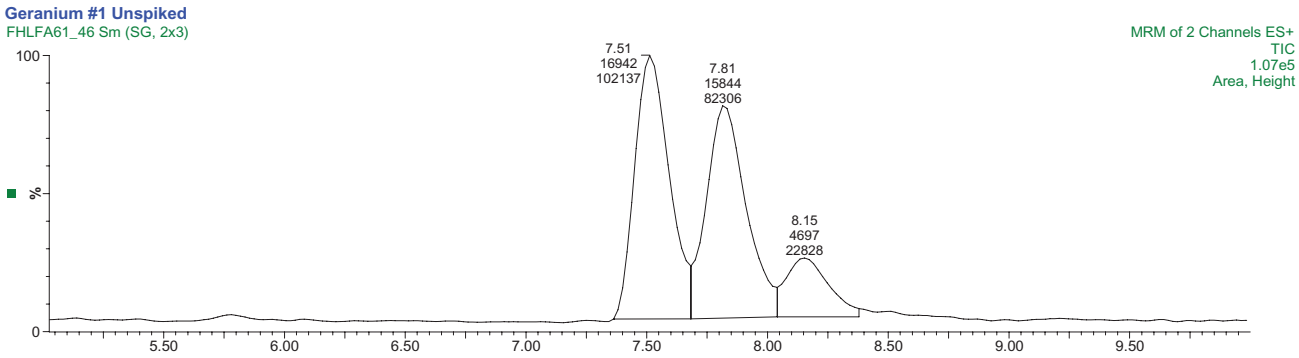


Figure S4. Analysis set 1—Changzhou S11-1, unspiked.

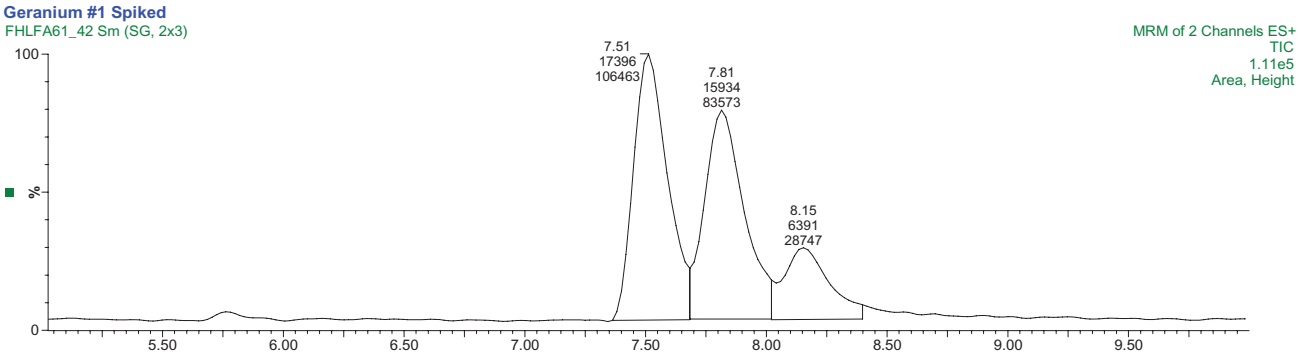


Figure S5. Analysis set 1—Changzhou S11-1, spike 10 μg/L.

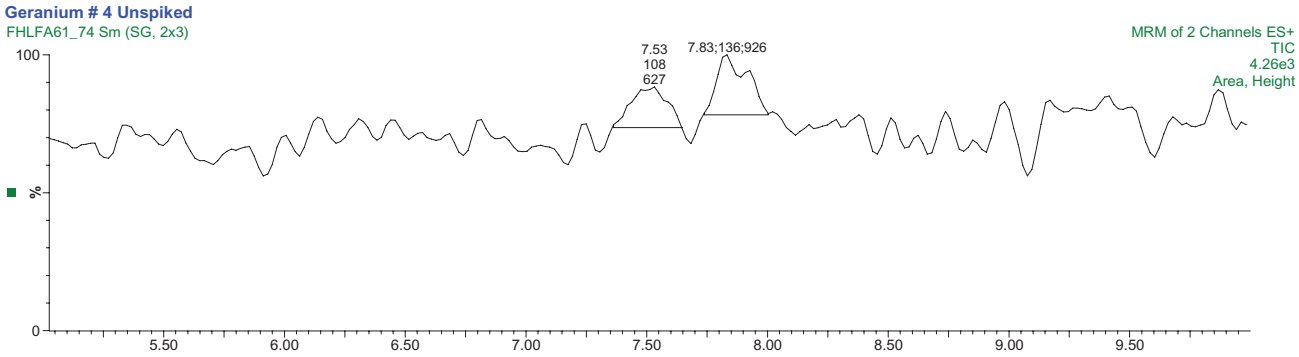


Figure S6. Analysis set 1—Guiyang 1, unspiked.

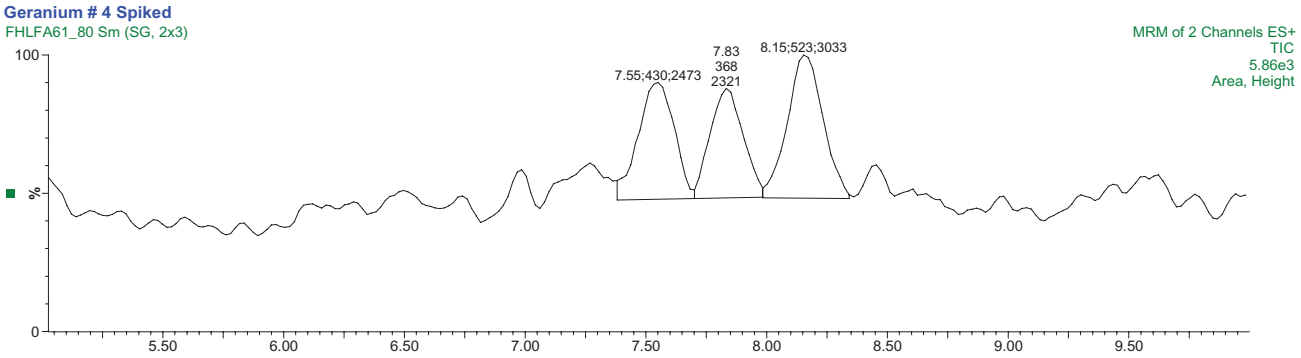


Figure S7. Analysis set 1—Guiyang 1, spike 10 μg/L.

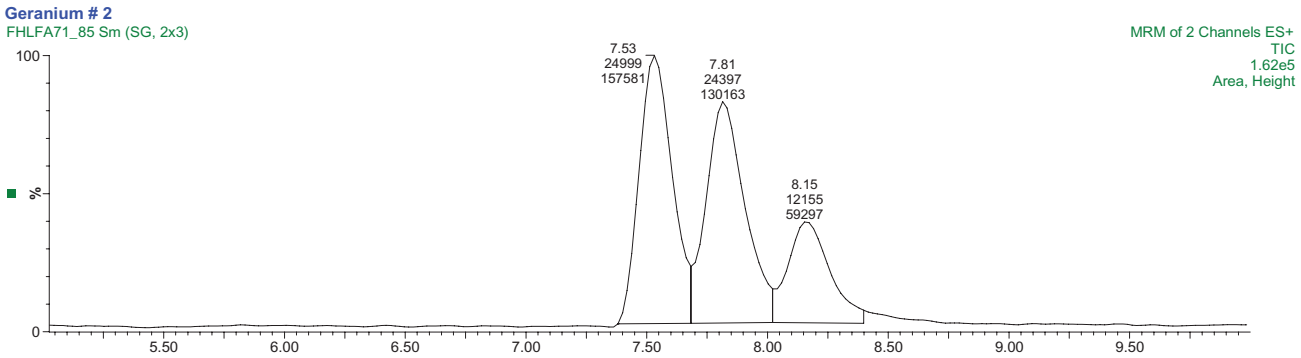


Figure S8. Analysis set 1—Changzhou 1, unspiked.

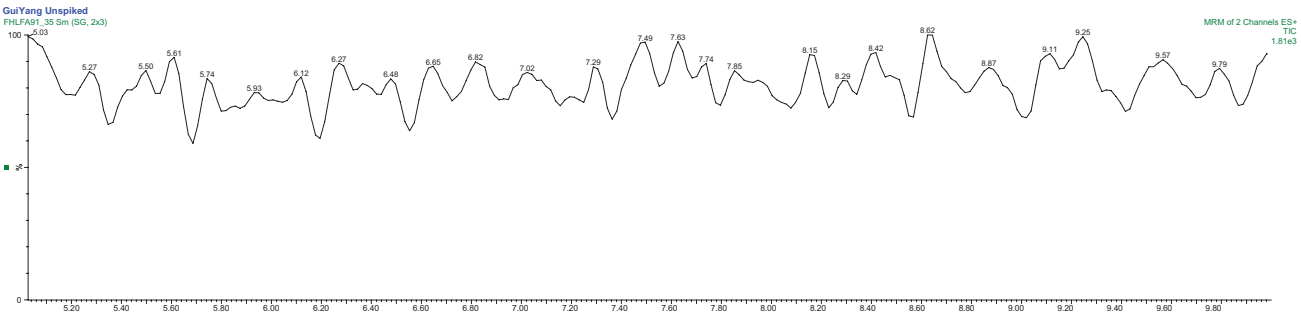


Figure S9. Analysis set 2—Guiyang 2 unspiked.

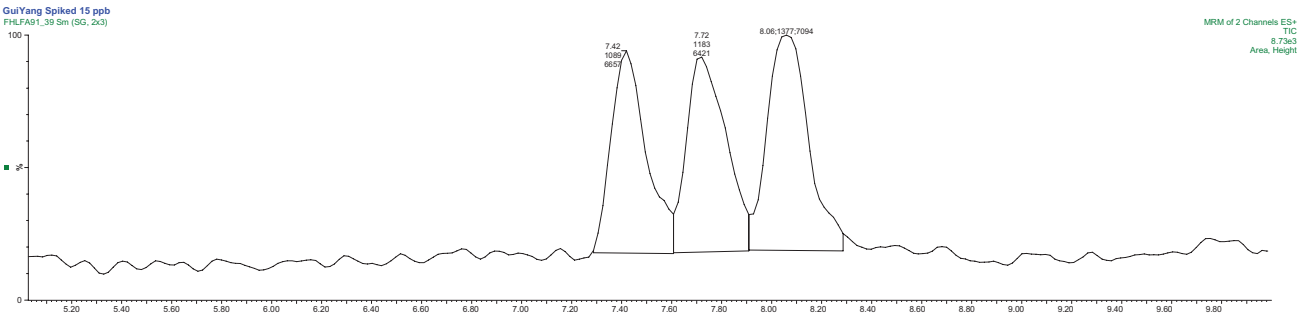


Figure S10. Analysis set 2—Guiyang 2, spike 15 μg/L.

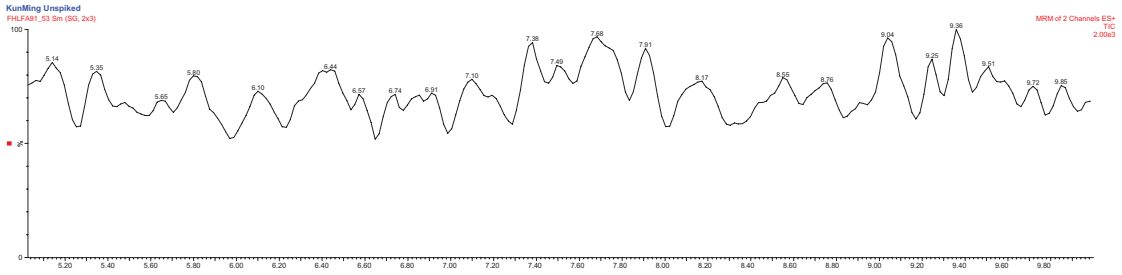


Figure S11. Analysis set 2—Kunming 2, unspiked.

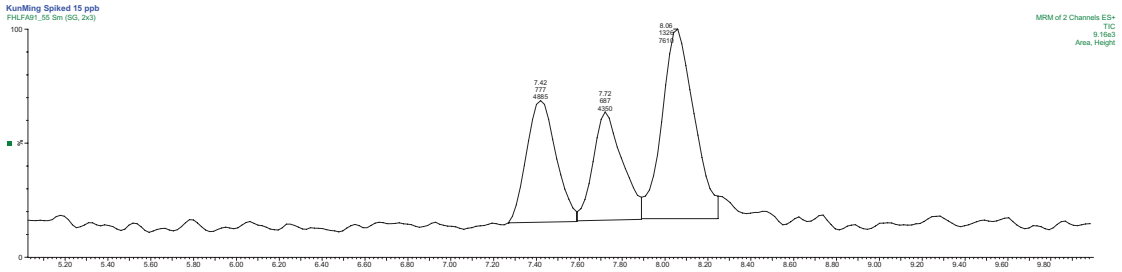


Figure S12. Analysis set 2—Kunming 2, spike 15 μg/L.

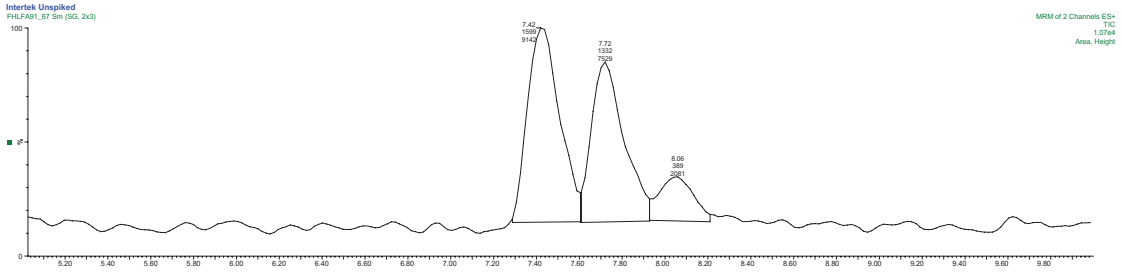


Figure S13. Analysis set 2—Changzhou S11-2 unspiked.

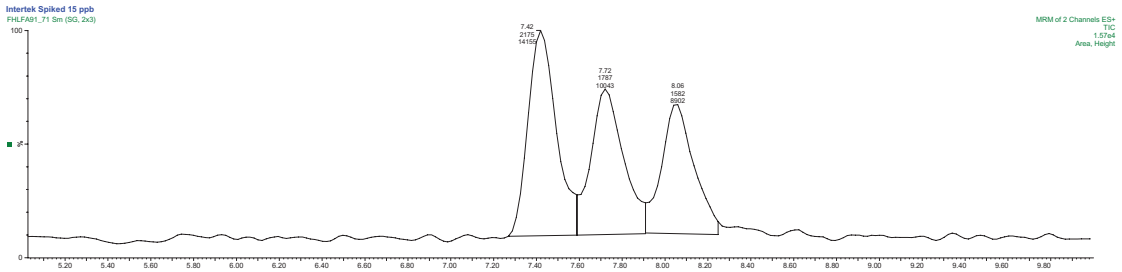


Figure S14. Analysis set 2—Changzhou S11-2, spike 15 μg/L.

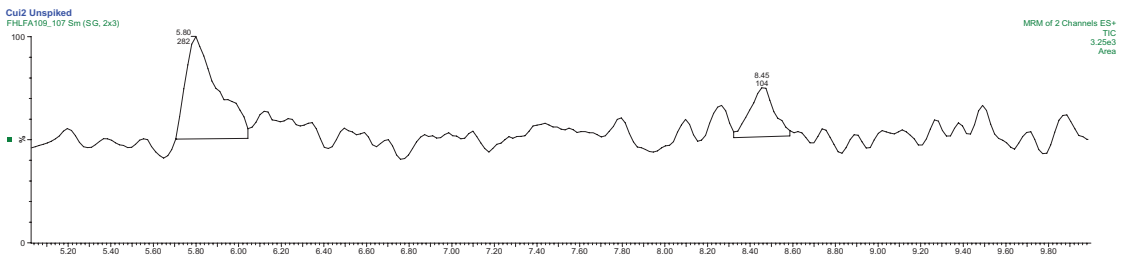


Figure S15. Analysis set 3—Guiyang 3, unspiked.

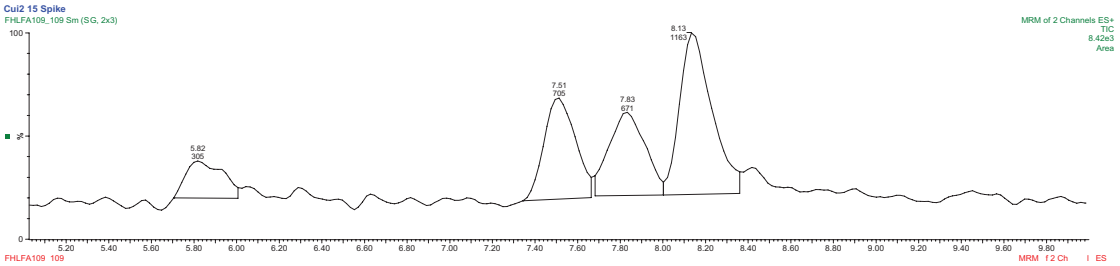


Figure S16. Analysis set 3—Guiyang 3, spiked 15 μg/L.

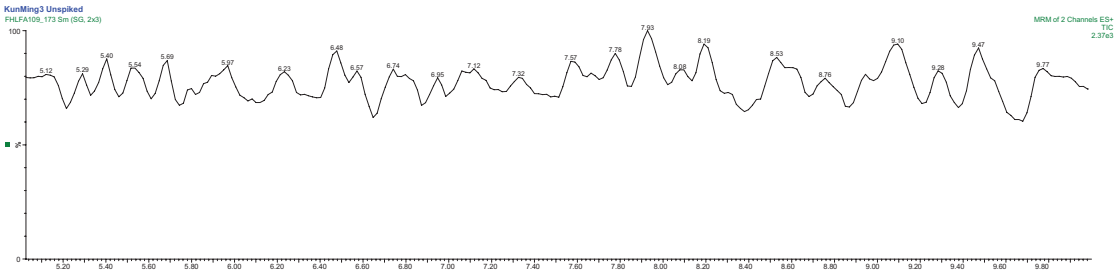


Figure S17. Analysis set 3—Kunming 3, unspiked.

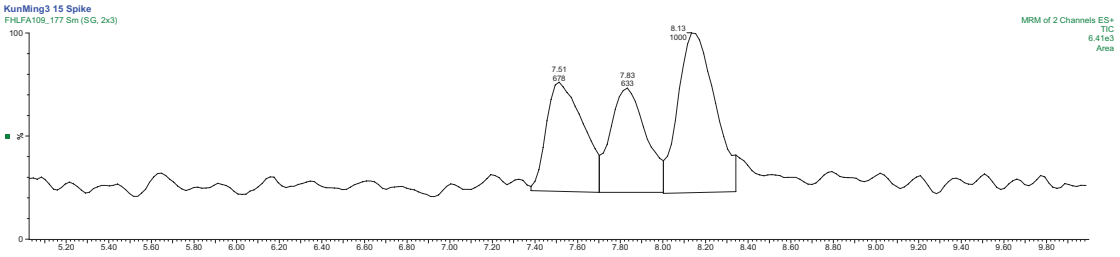


Figure S18. Analysis set 3—Kunming 3, spiked 15 μg/L.

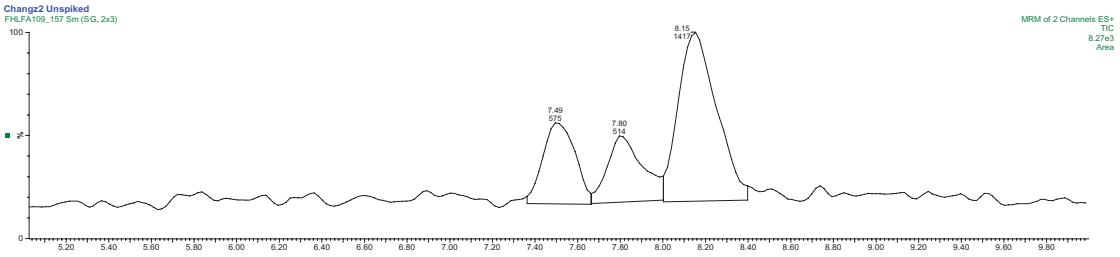


Figure S19. Analysis set 3—Changzhou 3, unspiked.

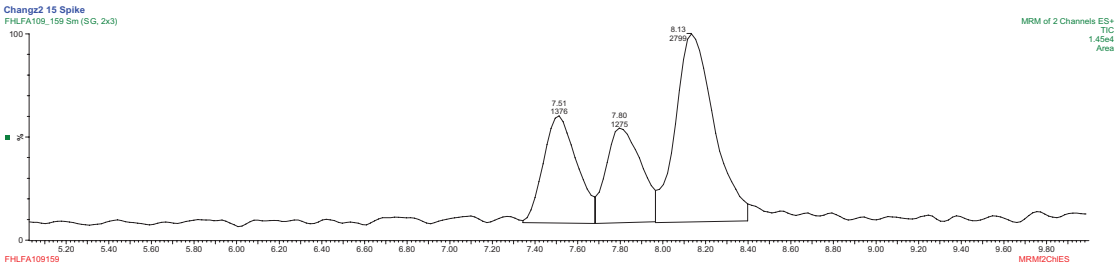
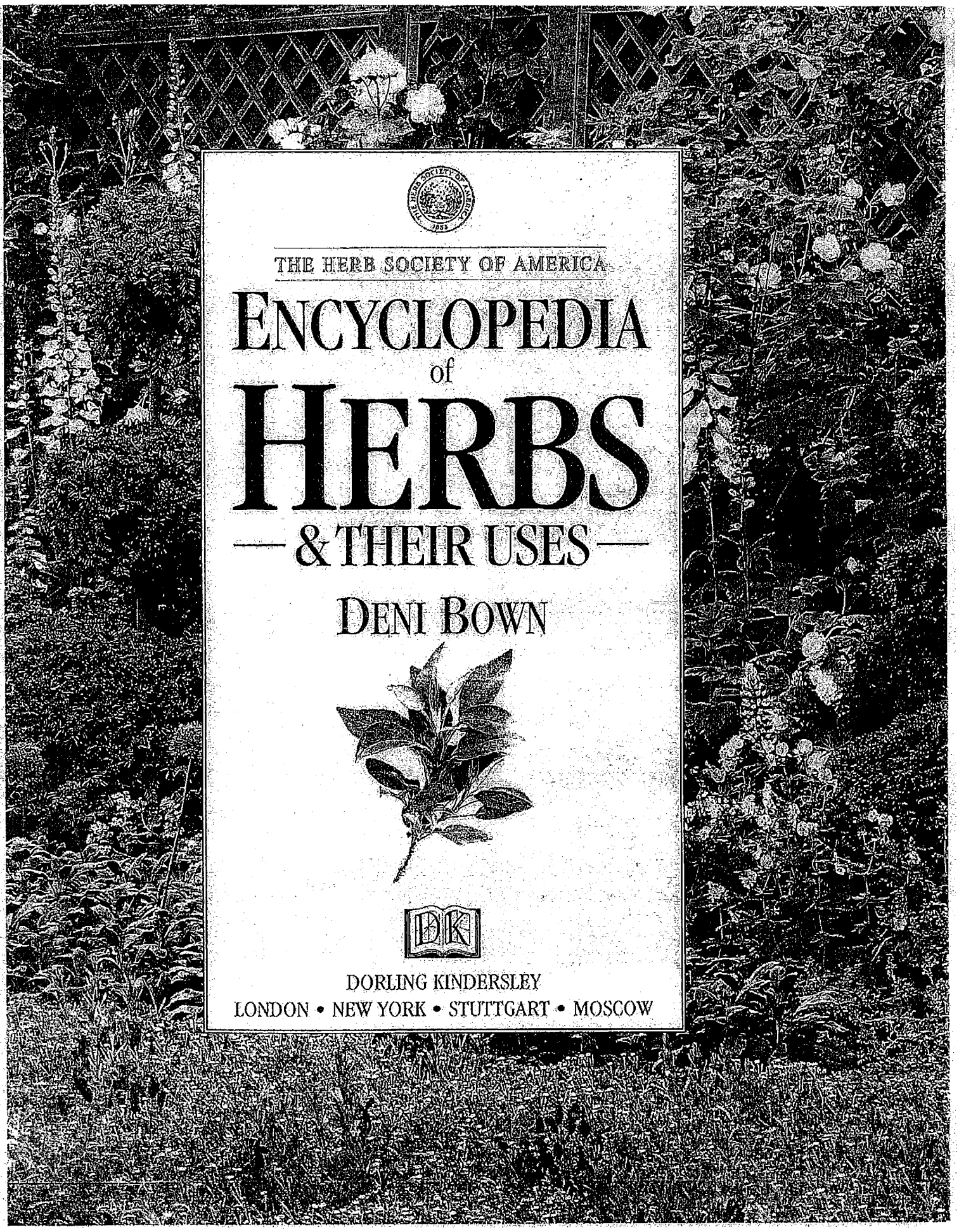


Figure S20. Analysis set 3—Changzhou 3, spiked 15 μg/L.

Supplementary data

A video abstract by the authors of this paper is available. video-abstract10445.mov



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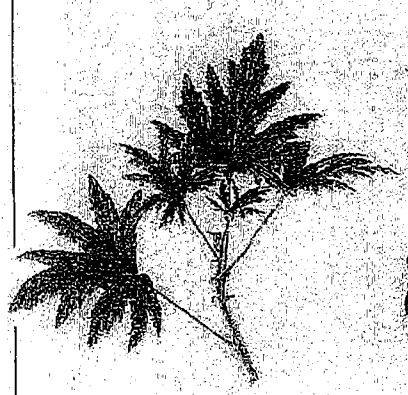
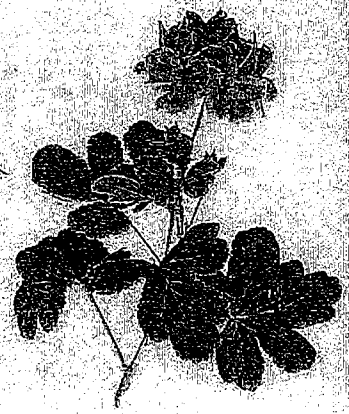
CONTENTS

PELARGONIUM

This genus contains about 250 species of annuals, perennials, and subshrubs, which occur mainly in S Africa, with a few in tropical Africa, the Middle East, and Australia. Pelargoniums are commonly known as geraniums, confusing them with members of the related genus, *Geranium* (see p.133). Scented geraniums have been cultivated in Europe since the 17th century, and numerous hybrids and cultivars exist. They have become popular in recent years for their intensely aromatic leaves and subtle coloring. They are quite different in appearance and uses from the more popular zonal, regal, and ivy-leaved geraniums, commonly grown for summer display. The true species of *P. capitatum* has been superseded by the hybrid *P.* 'Attar of Roses', which has a stronger scent and more upright habit. The name *Pelargonium* comes from the Greek *pelargos*, "stork," because the shape of the fruit resembles a stork's beak.



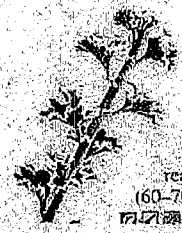
Pelargonium citronellum
An upright, bushy species with strongly lemon-scented leaves about 3½in (8cm) wide. Clusters of 5–8 purple-pink flowers with dark purple markings appear in summer. H 4–6ft (1.2–2m), S 3ft (1m).



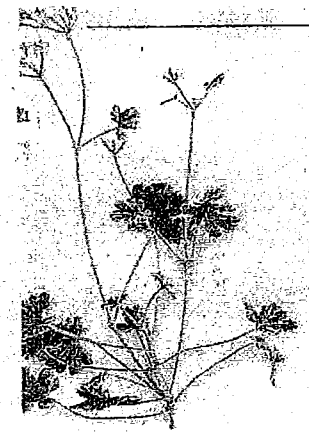
Pelargonium 'Clorinda'
Early 20th-century hybrid with a cedar-rose scent, bright green, round-lobed leaves about 2½in (6cm) long, and bright rose-pink flowers, 1½in (4cm) across. H and S 4ft (1.2m).

Pelargonium odoratissimum
(apple geranium)
Low-growing perennial with trailing flower stems and rounded, wavy-edged leaves which have a pronounced apple aroma. Small, white, red-veined flowers appear in spring and summer. H 12in (30cm), S 2ft (60cm).

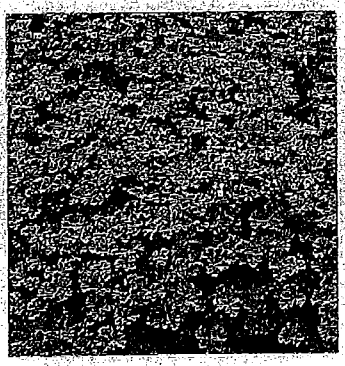
Pelargonium crispum 'Major'
This differs from the species in having larger leaves, up to 1in (2.5cm) long. The miniature version, *P. c.* 'Minor', has a stiff, compact habit. H 24–28in (60–70cm), S 12–18in (30–45cm).



Pelargonium crispum 'Variegatum'
A favorite cultivar with crinkled, cream-edged leaves and the same lemon scent as the species. Can be trained as a standard, reaching 3ft (1m). H 24–28in (60–70cm), S 12–18in (30–45cm).

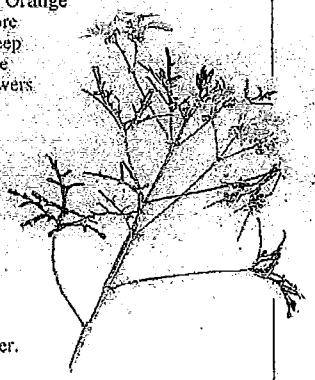


elargonium 'Fragrans
iegatum'
attractive silky leaves have
ular cream margins, which age to
green; they have the same nutmeg-
aroma as 'Fragrans'. H and
-16in (30–40cm).

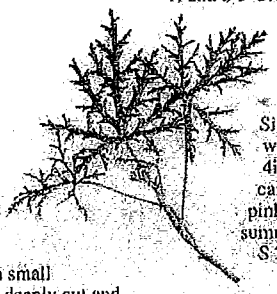


Pelargonium 'Fragrans'
(nutmeg geranium)
Erect subshrub with gray-green, silky, rounded leaves, which have a spicy, pinelike aroma. White flowers, about ⅛in (1cm) across marked with 2 red lines, are borne in spring and summer. H and S 18in (45cm).

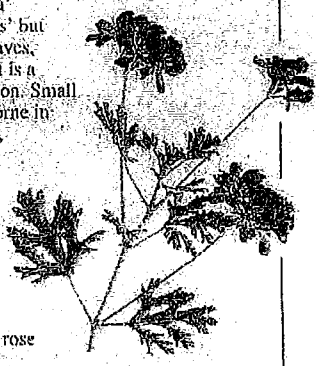
Pelargonium 'Prince of Orange'
Derived from *P. crispum* before 1880, this has a compact habit, deep green, fan-shaped leaves, and pale mauve-pink, purple-veined flowers reaching 1¼in (3cm) long. H and S 24in (60cm).



Pelargonium radens (rasp-leaved geranium)
Tall, bushy subshrub, introduced to the UK in 1774. It has a pungent lemon aroma and rough, finely divided leaves. Pale purple-pink flowers appear in summer. H and S 3–5ft (1–1.5m).

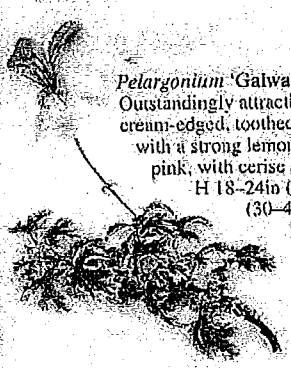


Pelargonium 'Radula'
Similar to *P.* 'Graveolens' but with more deeply cut leaves, 4in (10cm) across. Scent is a camphoraceous rose-lemon. Small pink-purple flowers are borne in summer. H 3–5ft (1–1.5m), S 2–5ft (60cm–1.5m).



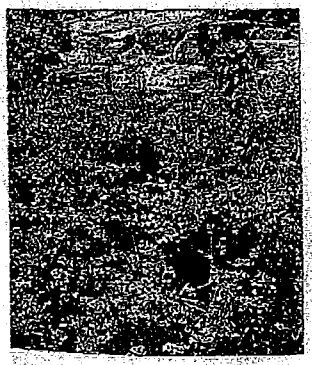
Pelargonium 'Radula Rosea'
This cultivar makes a colorful pot plant with deep rose pink flowers. H 3–5ft (1–1.5m), S 2–5ft (60cm–1.5m).

Pelargonium 'Graveolens'
e geranium)
ight subshrub with gray-green, rose-
ated, triangular, toothed leaves up to
n (6cm) long. Pale pink flowers with
purple spots are borne in spring and
mer. Dates back to the 1790s.
–5ft (1–1.5m), S 2–5ft (60cm–1.5m).



Pelargonium 'Galway Star'
Outstandingly attractive, with small cream-edged, toothed leaves, deeply cut and with a strong lemon scent. Flowers are pale pink, with cerise and purple markings. H 18–24in (45–60cm), S 12–18in (30–45cm).

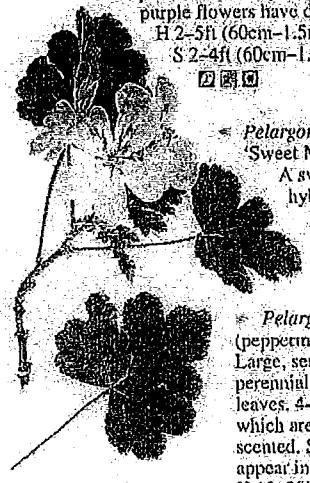
Pelargonium 'Rober's Lemon Rose'
Tall, vigorous variant of *P.* 'Graveolens' with soft, gray-green, irregularly lobed leaves about 2in (5cm) long. It has a lemon-rose scent and small pink flowers. H 5ft (1.5m), S 4ft (1.2m).



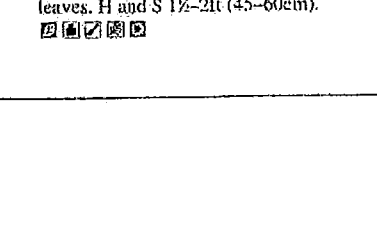
Pelargonium capitatum
ild rose geranium)
w, spreading, evergreen, perennial
h velvety, crinkled, rose-scented
ves up to 3in (8cm) long. Mauve-pink
wers, ¾in (2cm) across, are borne in
sters in summer. H 1–3ft (30–90cm),
½–5ft (45cm–1.5m).

Pelargonium 'Lady Plymouth'
First recorded about 1800, this hybrid's triangular leaves have irregular cream margins and a minty rose-lemon scent. 'Grey Lady Plymouth' has mainly gray-green foliage. H 3–5ft (1–1.5m), S 2–5ft (60cm–1.5m).

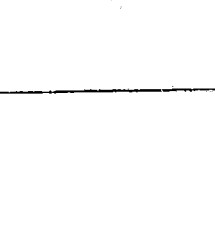
Pelargonium 'Royal Oak'
A shrubby, balsam-scented hybrid with slightly sticky leaves, 2–3in (5–7cm) long, which resemble an oak leaf in shape. The relatively large pink-purple flowers have darker spots. H 2–5ft (60cm–1.5m), S 2–4ft (60cm–1.2m).



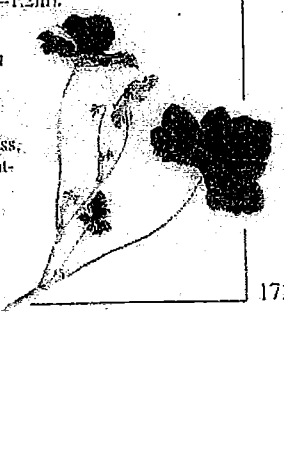
Pelargonium 'Sweet Mimosa'
A sweetly scented hybrid with bright green, round-lobed leaves about 3½in (9cm) across, and bright shell pink flowers. H and S 3–4ft (1–1.2m).



Pelargonium 'Old Spice'
A cultivar derived from *P.* 'Fragrans', with a compact habit and crinkled leaves. H and S 1½–2ft (45–60cm).



Pelargonium tomentosum
(peppermint geranium)
Large, semiprostrate, shrubby perennial with soft, velvety leaves, 4–5in (10–12cm) across, which are strongly peppermint-scented. Small white flowers appear in spring and summer. H 12–20in (30–50cm), S 3–4ft (1–1.2m).



SSIFLORA Passionflower
sifloraceae)

ish missionaries in S America regarded flowers as symbols of Christ's passion – ree stigmas representing the nails, the nthers the wounds, and the ten sepals postles (Peter and Judas Iscariot being it). *P. incarnata* was used in native nerican medicine, notably by the Houma who added it to drinking water as a . It was described by a visiting European r in 1783 as a remedy for epilepsy and ne a popular treatment for insomnia in 9th century, with an entry in the U.S. *nal Formulary* (1916–36). The herb ins alkaloids and flavonoids, which are ive, nonaddictive sedatives that do not drowsiness. One flavonoid, apigenin, is tispasmodic and anti-inflammatory, and s in various unrelated plants, notably *n graveolens* (see p.240).

carnata (maypops, onflower) p.171

USED Whole plant, fruits.
RTIES A bitter, sedative, cooling herb that s pain, relaxes spasms, and lowers blood re.
F THE HERB
RY Fruits are pulped for jam and desserts.
NAL Internally for nervous tension, insomnia, lity, tension headache, asthma, irritable syndrome, premenstrual tension, nervous ardia, hypertension, and shingles. Combines ith *Valeriana officinalis* (see p.367), *emelum nobile* (see p.259), and *Humulus* (see p.294) for insomnia. Not nended during pregnancy.

GROWTH AND HARVEST
WTH Crop. Well-drained, sandy, slightly soil in sun. Propagate by seed sown in g. at 18–21°C (64–70°F), or by semiripe ngs in summer. Germination is slow and c. Cut back in early spring. Cucumber ic virus may attack leaves. The fruits only in warm, sunny climates.
VEST Plants are cut when fruiting and dried se in infusions, liquid extracts, tablets, and ures. Fruits for culinary use are picked ripe in autumn and used fresh or cooked.

LLINIA
idaceae)

of *P. cupana* are used by the Guaranis zilian Amazonia to make a stimulant much as tea and coffee are. They n up to seven percent of a caffeinelike und, known as "guaranine." Unlike e, it is not addictive and takes longer metabolized, giving it a gentler, more ed stimulant effect. It has recently been ted as a safe, natural stimulant by the food industry in the form of capsules idy, and as a component of tonic based on spring water, fruit juices, and herbal extracts. Stems of the closely related *P. yoco* (yoco) are used by native people in Colombia, Ecuador, and northern Peru to make a similar drink.

P. cupana, syn. *P. sorbilis* (guaranà, Brazilian cocoa, zoom) p.171

PARTS USED Seeds.
PROPERTIES An astringent, bitter herb with a strong stimulant effect.
USES OF THE HERB
CULINARY Seeds are roasted, ground, and pressed into paste (*pasta guaranà*), which is dried into sticks and then grated into water as a tealike drink.
MEDICINAL Internally to relieve fatigue, aid concentration, and lift the spirits. May cause sleeplessness, although reputedly less so than caffeine-based products. Not recommended for those with cardiovascular disease or hypertension.
ECONOMIC Added to diet foods, supplements for athletes, tonic drinks, and chewing gum. Seeds also fermented locally to make an alcoholic drink. Used as a source of caffeine and flavoring for soft drinks, liqueurs, and candy. Sweetened paste is known as "Brazilian chocolate."

GROWTH AND HARVEST
GROWTH Crop. Moist, humus-rich soil in partial shade, minimum 65°F (18°C). Propagate by ripewood cuttings at the end of the growing season.
HARVEST Seeds are collected when ripe, then roasted, ground, and stored as paste or powder.

PAUSINYSTALIA
(Rubiaceae)

This western African genus consists of 13 species of large trees characterized by panicles of tubular flowers with conspicuous appendages. Medicinal use of yohimbe bark appears to have reached Europe in the 1890s. *P. yohimbe* and the related *P. macroceras* and *P. tillesii* contain indole alkaloids, the principal one being yohimbine, which blocks the release of adrenalin and acts as a sexual stimulant. In Africa *P. lane-poolei* (*pamprana*, *igbepo*) is also used medicinally; dressings of ground bark are applied to yaws (a skin infection) and itching skin.

P. yohimbe, syn. *Corynanthe yohimbe* (*yohimbe*, *endone*)

Tender evergreen tree, height 90ft (27m), spread 40ft (12m), with red to yellow-ochre wood and glossy, oblanceolate, dark green leaves up to 14in (35cm) long. White or pink tubular flowers appear in clusters arranged in panicles up to 7in (18cm) long in winter in the wild, followed by capsules containing small, winged seeds.

PARTS USED Bark.
PROPERTIES A bitter, warming, antidiuretic herb with reputedly aphrodisiac effects. It has a stimulant effect on the heart, increases heart rate and blood pressure, and is locally anesthetic.
USES OF THE HERB
MEDICINAL Internally for impotence and frigidity. Interacts with certain antihypertension drugs. Not given to patients with hypertension, or renal or hepatic disease. Excess may cause depression.
WARNING This herb is subject to restrictions in some countries.

GROWTH AND HARVEST
GROWTH Wild-collected. Moist soil with high humidity, minimum 59–64°F (15–18°C). *P. yohimbe* does not appear to be in cultivation, and no information has been found on its requirements.
HARVEST Bark is dried in strips for pills, liquid extracts, and extraction of yohimbine.

PEGANUM
(Zygophyllaceae)

P. harmala is much used in Arab medicine and is mentioned in early Muslim medical literature. It contains hallucinogenic alkaloids and has a history of use in folk medicine.

P. harmala (Syrian rue, harmal) p.174

PARTS USED Fruits, seeds, oil.
PROPERTIES A bitter, spicy, diuretic herb that stimulates the uterus and digestive system, and is reputedly aphrodisiac.
USES OF THE HERB
CULINARY Seeds are used as a spice and purifying agent.
MEDICINAL Locally, internally for stomach complaints, urinary and sexual disorders, epilepsy, menstrual problems, nervous and mental illnesses. Excess causes hallucinations and vomiting. Externally for hemorrhoids and baldness.
ECONOMIC Fruits yield a red dye and oil.

GROWTH AND HARVEST
GROWTH Crop. Well-drained to dry, poor soil in sun. Propagate by seed sown in late spring, or by division in late spring. Subject to statutory control as a weed in parts of Australia.
HARVEST Fruits and seeds are collected when ripe. Fruits are pressed for dye and oil. Seeds are dried for use in infusions and ointments.

PELARGONIUM Geranium
(Geraniaceae)

Scented geraniums contain very complex volatile oils. Over 2,000 components have been found, including those with discernible similarities to orange, lemon, peppermint, rose, nutmeg, and eucalyptus. Most popular are the rose-scented species and cultivars such as *P.* 'Rober's Lemon Rose'. They yield geranium oil, which is a fragrance in its own right and is often used as an adulterant of attar of roses. *P. capitatum* and cultivars related to *P. graveolens* and 'Radula' are grown for the production of geranium oil in parts of France, Italy, India, Egypt, Algeria, and the former Soviet Union. The finest, known as "Bourbon oil," comes from the island of Réunion. Many species are used medicinally in southern Africa, mainly for digestive, bronchial, and skin problems. *P. betulinum* (camphor geranium, birch-leaf geranium) is a good

decongestant and digestive; leaves of *P. antidysentericum* (dysentery geranium) are infused and taken with lemon juice for gastrointestinal complaints. The leaves of *P. acetosum* (sorrel leaf) have an acidic taste and may be eaten raw in salads or added to soups and stews; several species, such as *P. rapaceum* and *P. triste*, have tuberous roots that are cooked like potatoes. Most species are easily propagated and hybridized, and the feasibility of producing aromas for the food and perfumery industries from plant cells grown *in vitro* has recently been examined.

P. capitatum (wild rose geranium) p.173

PARTS USED Whole plant, leaves, oil.
PROPERTIES An aromatic, soothing, emollient herb with a roselike aroma.
USES OF THE HERB
AROMATIC Oil is used in perfumery. Leaves are dried for potpourris.
MEDICINAL Internally as a traditional Cape remedy for minor digestive ailments and kidney and bladder disorders. Externally for rashes and calloused and cracked skin. Oil is a major component of geranium oil, used in aromatherapy and skin care.

P. citronellum p.172

PARTS USED Leaves.
PROPERTIES An aromatic herb with a lemon scent.
USES OF THE HERB
CULINARY Leaves may be infused to make tea and used fresh to flavor desserts, punch, and vinegar.
AROMATIC Leaves are dried for potpourris and herb pillows.

P. 'Clorinda' p.172

PARTS USED Leaves.
PROPERTIES An aromatic herb with a cedar–rose perfume.
USES OF THE HERB
AROMATIC Mainly grown as an aromatic ornamental. Leaves may be dried for potpourris.

P. crispum (lemon geranium) p.172

PARTS USED Leaves.
PROPERTIES An aromatic herb with a lemon aroma.
USES OF THE HERB
CULINARY Leaves may be infused to make tea and used fresh to give a lemon flavor to sauces, sorbets, ice cream, cakes, fruit punch, and vinegar.
AROMATIC Leaves are dried for potpourris and herb pillows.

USES OF THE HERB
Leaves may be used as for *P. crispum*.

P. 'Fair Ellen' p.172

PARTS USED Leaves.
PROPERTIES An aromatic herb with a balsamlike scent.
USES OF THE HERB
AROMATIC Mainly grown as an aromatic ornamental. Leaves may be dried for potpourris.

P. 'Fragrans' (nutmeg geranium) p.173

PARTS USED Leaves.
PROPERTIES An aromatic herb with a spicy scent.
USES OF THE HERB
AROMATIC Mainly grown as an aromatic ornamental. Dried leaves are added to potpourris.
MEDICINAL Externally as a rub for aching feet or legs (fresh leaves).
ECONOMIC Leaves give flavor to *pâté* and a spicy flavor to coffee.

P. 'Fragrans Variegatum' p.173

PARTS USED Leaves.
PROPERTIES An aromatic herb with a spicy scent.
USES OF THE HERB
Leaves may be used as for *P.* 'Fragrans'.

P. 'Galway Star' p.173

PARTS USED Leaves.
PROPERTIES An aromatic herb with a lemon scent.
USES OF THE HERB
Leaves may be used as for *P. crispum*.

P. graveolens (rose geranium) p.173

PARTS USED Whole plant, leaves, oil.
PROPERTIES An aromatic, rose-scented herb that has relaxant, antidepressant, and antiseptic effects; reduces inflammation, and controls bleeding.
USES OF THE HERB
CULINARY Fresh leaves are infused for tea and used in similar ways to those of *P. crispum*.
AROMATIC Oil is used in perfumery and aromatherapy. Dried leaves added to potpourris.
MEDICINAL Internally for premenstrual and menopausal problems; nausea, tonsillitis, and poor circulation. Externally for acne, hemorrhoids, eczema, bruises, ringworm, and lice.
ECONOMIC Oil is the main constituent of geranium oil, used in skin care and food flavoring.

P. 'Lady Plymouth' p.173

PARTS USED Leaves.

P. odoratissimum (apple geranium)

PARTS USED Whole plant, leaves, oil.
PROPERTIES An aromatic herb with a fruity scent. It has astringent, tonic, and antiseptic effects, controls bleeding, promotes healing, and repels insects.
USES OF THE HERB
CULINARY Leaves may be used as for *P. crispum*.
AROMATIC Leaves may be used as for *P. crispum*.
MEDICINAL Internally for debility, gastroenteritis, and hemorrhage. Externally for skin complaints, injuries, neuralgia, and throat infections. Oil used in aromatherapy for burns, sores and shingles.

P. 'Old Spice' p.173

PARTS USED Leaves.
PROPERTIES An aromatic herb with a spicy scent.
USES OF THE HERB
Leaves may be used as for *P.* 'Fragrans'.

P. 'Prince of Orange' p.173

PARTS USED Leaves.
PROPERTIES An aromatic herb with a citrus scent.
USES OF THE HERB
Leaves may be used as for *P. crispum*.

P. quercifolium (oak-leaved geranium)

Tender, erect subshrub, height 1½–5ft (45cm–1.5m), spread 12–36in (45–90cm), with rough-textured, balsam-scented leaves, 2–4in (5–10cm) long, which are triangular in outline and deeply divided. Purple-pink flowers with darker markings appear in spring and summer.
PARTS USED Leaves.
PROPERTIES An aromatic, stimulant herb with a resinous scent.
USES OF THE HERB
AROMATIC Dried leaves are added to potpourris.
MEDICINAL Internally as a Cape remedy for rheumatism, hypertension, and heart disease.
ECONOMIC Dried leaves are added to insect-repellent sachets.

P. radens (rasp-leaved geranium) p.173

PARTS USED Leaves.
PROPERTIES An aromatic herb with a lemony scent.
USES OF THE HERB
MEDICINAL Externally as a rub for aching feet or legs (fresh leaves).
ECONOMIC Dried leaves are added to insect-repellent sachets and act as a fixative for other perfumes in potpourris.

P. 'Radula' p.173

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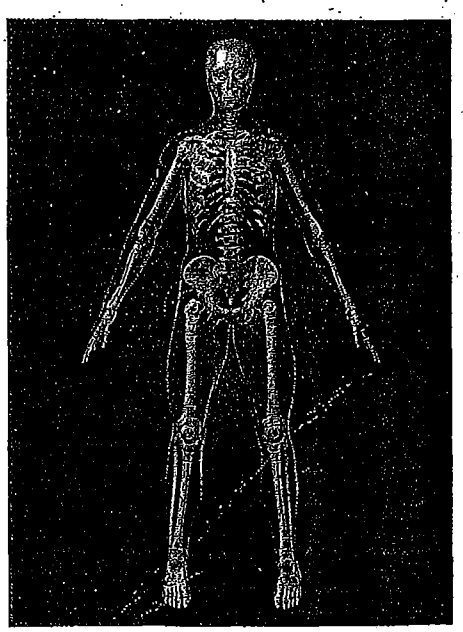

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Effects of 1,3-Dimethylamylamine and Caffeine Alone or in Combination on Heart Rate and Blood Pressure in Healthy Men and Women

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Richard J. Bloomer, PhD[1]
Innocence C. Harvey, BS[1]
Tyler M. Farney, MS[1]
Zach W. Bell, BS[1]
Robert E. Canale, MS[1]

[1]Cardiorespiratory/Metabolic Laboratory, Department of Health and Sport Sciences, University of Memphis, Memphis, TN

Abstract

Background: The use of 1,3-dimethylamylamine (geranamine), alone and in combination with caffeine, is becoming widespread within the dietary supplement industry. To our knowledge, no data are available concerning the effects of oral geranamine intake on heart rate (HR) and blood pressure in individuals. **Methods:** Ten young healthy men and women ingested 1 of 5 conditions on different days using a double-blind, randomized, crossover design. The following were ingested after a 10-hour overnight fast: 250 mg caffeine (C), 50 mg geranamine (G 50 mg), 75 mg geranamine (G 75 mg), 250 mg caffeine + 50 mg geranamine (C + G 50 mg), and 250 mg caffeine + 75 mg geranamine (C + G 75 mg). Heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), and rate pressure product (RPP) were measured pre-ingestion and at 30, 60, 90, and 120 minutes post-ingestion. Plasma norepinephrine (NE) and epinephrine (EPI) were measured pre-ingestion and at 60 and 120 minutes post-ingestion. **Results:** Heart rate was unaffected by treatment, but blood pressure and RPP were higher with geranamine, generally in a dose-dependent manner. The peak percent change from pre-ingestion in SBP (~20%), DBP (~17%), and RPP (~9%) was noted with C + G 75 mg at 60 minutes post-ingestion. Plasma NE and EPI were relatively unaffected by treatment. **Conclusion:** We report for the first time that acute ingestion of 1,3-dimethylamylamine alone and in combination with caffeine results in an increase in SBP, DBP, and RPP without an increase in HR. The largest increase is observed at 60 minutes post-ingestion of C + G 75 mg. These changes cannot be explained by circulating NE and EPI.

Keywords: caffeine; geranamine; blood pressure; heart rate; catecholamines

Introduction

Regardless of economic conditions, dietary supplements are a multimillion-dollar-per year industry, according to the *Nutrition Business Journal*.[1] Many products contain natural (ie, herbal) and synthetic stimulants, such as guarana, ma huang, Kola nut, phenylethylamine, and caffeine. One recent addition to the stimulant category is geranium extract, which has been noted to be present in small amounts in geranium oil.[2] This ingredient has received a great deal of attention in recent years and is included in multiple dietary supplements targeting weight/fat loss, as well as enhanced physical performance. Although anecdotal reports of improved focus and mood, enhanced exercise performance, and decreased appetite are common, to our knowledge, no studies have attempted to verify these claims using controlled laboratory procedures. Moreover, while only scant data are available pertaining to the hemodynamic effects of intravenous and nasal inhalation delivery of this ingredient in animals[3,4] and humans (as noted in documentation from Eli Lilly & Co. regarding methylhexamine [Forthane™]),

Correspondence: Richard J. Bloomer, PhD, Department of Health and Sport Sciences, The University of Memphis, 161F Roane Field House, Memphis, TN 38152.
Tel: 901-678-4341
Fax: 901-678-3591
E-mail: rbloomer@memphis.edu

respectively, no studies to our knowledge have determined the effect of acute oral intake of geranium extract on heart rate (HR) and blood pressure in human subjects. This is important information to obtain considering the widespread use of this ingredient in many dietary supplements that are currently sold on the worldwide market.

A review of information obtained from various online sources, including 1 scientific article,[2] indicates that geranium extract may be a component of the *Pelargonium graveolens* plant, with anecdotal health benefits noted on various Web sites, as well as antioxidant[5] and other potential health benefits noted in the scientific literature.[6] In much the same way as caffeine is associated with both positive[7-10] and potentially negative[11-15] effects on human health and function, the same may be true for geranium extract.

The action of geranium extract appears as a simple aliphatic amine functioning as a norepinephrine (NE) reuptake inhibitor and/or NE-releasing agent. According to patent data available via the US Patent and Trademark Office (document 2,350,318) entitled "Aminoalkanes" (dated April 9, 1942), geranium extract (noted as 2-amino-4-methylhexane) stimulates smooth muscle and may act as a vasoconstrictor. In addition to 2-amino-4-methylhexane, other common chemical names of this agent include 1,3-dimethylamylamine, 1,3-dimethylpentylamine, methylhexaneamine, 4-methyl-2-hexylamine, and geranamine (Proviant Technologies, Inc., Champaign, IL), which was trademarked in 2005 (US trademark number: 78542697).

Aside from the above information and the anecdotal reports from individuals using geranamine, very little is known about this ingredient, despite its widespread availability and use. Therefore, the purpose of this investigation was to determine the effect of geranamine intake at 2 different dosages of practical relevance, with and without the addition of caffeine (as caffeine is commonly combined with geranamine in many dietary supplements), on HR and blood pressure. We included a caffeine-only condition for comparison purposes, as the effects of caffeine on HR and blood pressure are well described.[16] Based on the potential vasoconstrictor properties of geranamine, we also measured plasma NE and epinephrine (EPI) in response to treatment. The study involved a single oral serving of each condition by healthy men and women, with observation for a 2-hour post-ingestion period.

Materials and Methods

Subjects

Young, healthy, exercise-trained men (n = 5) and women (n = 5) participated in this investigation. All subjects completed a medical history and physical activity questionnaire in order to determine eligibility. No subject was a smoker, used smokeless tobacco products, or had diagnosed cardiovascular (eg, hypertension) or metabolic disease. Six subjects reported using caffeine daily through consumption of coffee (n = 5) or soda (n = 1). The mean intake for these 6 subjects was estimated at 220 mg per day. Three other subjects reported occasional use of caffeine through consumption of coffee or soda (eg, once per week). One subject reported never using caffeine. Men and women were considered exercise trained, as they performed combined aerobic and anaerobic exercise for 6 ± 3 and 7 ± 3 hours per week, respectively, for the past several years. Subject descriptive characteristics are presented in Table 1. All experimental procedures were performed in accordance with the Declaration of Helsinki. The University of Memphis Human Subjects Committee approved all experimental procedures (H10–45), and subjects provided verbal and written consent prior to participating in this study.

Testing and Conditions

All testing procedures described below were identical for all 5 test days. Subjects reported to the laboratory in a 10-hour fasted state, and all testing was completed in the morning hours. The time of testing was matched for subjects for all conditions. Subjects were instructed to not exercise for the 24 hours prior to each test day. On arrival to the laboratory, subjects were asked to void and then rest quietly for 10 minutes in a seated position. Following this quiet rest period, HR (via radial artery palpation for 60 seconds by 2 trained technicians) and systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured via auscultation using a dual earpiece stethoscope, and a blood

Table 1. Demographics of Study Participants

Variable	Men	Women
Age, y	26 ± 5	23 ± 3
Height, cm	174 ± 4	167 ± 7
Weight, kg	88 ± 11	73 ± 17
BMI, kg/m²	29 ± 3	26 ± 6
Body fat, %	16 ± 6	24 ± 8
Waist, cm	90 ± 9	76 ± 12
Hip, cm	106 ± 6	102 ± 10
Resistance exercise training, y	8 ± 7	4 ± 3
Resistance exercise, h/wk	5 ± 3	2 ± 1
Aerobic exercise training, y	3 ± 4	3 ± 3
Aerobic exercise,[a] h/wk	1 ± 1	5 ± 4

Data are mean ± standard deviation.
[a]No statistically significant differences noted in any variable ($P > 0.05$) except for hours per week aerobic exercise ($P = 0.05$).

sample was obtained pre-ingestion. Subjects were then provided their assigned condition and ingested it in the presence of an investigator. Heart rate, SBP, and DBP were recorded again at 30, 60, 90, and 120 minutes post-ingestion. Rate pressure product (RPP) was calculated as an indication of myocardial work, using the equation: HR × SBP. Blood was again obtained at 60 and 120 minutes post-ingestion. During and following each condition, subjects were asked to report any effects that were felt. Subjects rested quietly during the entire 2-hour period of data collection and consumed no food. However, water was allowed *ad libitum* and matched for subjects on the days of testing (mean intake, 517 mL).

The 5 conditions were provided in random order, using a double-blind, crossover design. The conditions were encapsulated, taken with water, and consisted of: 250 mg caffeine (C), 50 mg geranamine (G 50 mg), 75 mg geranamine (G 75 mg), 250 mg caffeine + 50 mg geranamine (C + G 50 mg), and 250 mg caffeine + 75 mg geranamine (C + G 75 mg). The dosages used in the present design are common for both caffeine and geranamine, as determined via current dietary supplement product labels and human subject reports assessed via various internet sites. The geranamine (1,3-dimethylamylamine HCL) was purchased from Waseta International Trading Co., Ltd. (Shanghai, China), and the caffeine (caffeine anhydrous) was purchased from Hi-Tech Pharmaceuticals, Inc. (Norcross, GA). Certificates of analysis for each ingredient indicated purity.

It should be noted that no placebo condition was included in the present design, which may be considered to be a limitation. However, we justify our omission of a placebo condition in the following manner. First, we know from our prior work and from the work of others that our measured variables are very stable in men and women when they are not receiving an active condition.[17–19] That is, little to no change in HR or blood pressure is noted when men and women simply rest quietly in a controlled environment. Second, our main objective in the present design was to determine the acute effect of geranamine alone and when combined with caffeine on HR and blood pressure, and to compute the percent change from pre-ingestion in these variables. We believe that the present design adequately provides these data. It should also be noted that the present design only attempted to investigate the acute (2-hour) effects of caffeine and geranamine alone or in combination, and at the dosages provided. Therefore, data from the present investigation do not provide information related to the effects of these conditions at times distant to 2 hours post-ingestion, or in regards to dosages less than or greater than what was provided here. Moreover, our data relate only to a single ingestion and do not provide clinical information related to long-term use of either ingredient alone or in combination—in particular in regards to the development of hypertension over time. It is possible that attenuation in response might be observed following long-term use, as a "tolerance" to regular treatment may be observed. This is known to occur in some but not all individuals who regularly consume caffeine—in terms of selective outcome measures.[20] Likewise, a tachyphylaxis has been demonstrated for geranamine.[3]

Blood Collection and Biochemistry

A total of 3 venous blood samples (7 mL per draw) were taken from subjects' forearm via needle and Vacutainer® (BD Diagnostics, Franklin Lakes, NJ). Blood was immediately processed in a refrigerated centrifuge to obtain plasma (4°C for 15 minutes at 2000 × g). Plasma samples were then stored at −70°C. Norepinephrine and EPI were determined using an enzyme-linked immunosorbent assay (2-CAT ELISA, BA 10–1500; Rocky Mountain Diagnostics, Colorado Springs, CO) following the instructions of the manufacturer (Labor Diagnostika Nord GmbH & Co., Nordhorn, Germany). In this competitive ELISA, NE and EPI were extracted by using a cis-diol–specific affinity gel, acylated, and then derivatized enzymatically. Standards were used to calculate unknown values of NE and EPI within each plasma sample, and controls were used to verify assay precision.

Statistical Analysis

Heart rate and blood pressure data were analyzed using a 5 (condition) × 5 (time) analysis of variance (ANOVA). Analysis of variance was also performed for change from baseline (pre-ingestion) for HR and blood pressure, and data are presented in figure format to provide a visual representation of the overall response for each variable measured. Epinephrine and NE data were analyzed using a 5 (condition) × 3 (time) ANOVA. Tukey's post-hoc tests were performed when necessary. Due to our small sample size and the novelty of this initial work focused on geranamine, no major attempt was made to determine sex-specific differences in the response to treatment. However, we did perform a correlation analysis (for each condition independently) on sex, body weight, and the percent change in SBP and DBP at 60 minutes post-ingestion (the time of peak response for most conditions). Statistical significance was set at $P \leq 0.05$. The analyses were done using JMP statistical software version 4.0.3 (SAS Institute, Cary, NC). Data are presented

at mean ± standard error of mean (SEM), except for subject characteristics, which are presented as mean ± standard deviation (SD).

Results

Subject Comments

All 10 subjects successfully completed all test days. The conditions were generally well tolerated; however, the following comments were provided by subjects: For C, 1 of 10 subjects reported feeling fatigued. For G 50 mg, 1 of 10 subjects reported feeling cold; 1 of 10 subjects reported feeling fatigued; and 2 of 10 subjects reported feeling lightheaded. For G 75 mg, 1 of 10 subjects reported that his/her nose and face felt "tingly." For C + G 50 mg, 1 of 10 subjects reported a lack of appetite and feeling very "awake." For C + G 75 mg, 1 of 10 subjects reported feeling lightheaded with somewhat labored breathing; 1 of 10 subjects reported that his/her ears felt "stopped up"; 1 of 10 subjects reported feeling "buzzed" with some chest tightness; 1 of 10 subjects reported that his/her nose had "cleared up" following ingestion.

Heart Rate and Blood Pressure Data (Absolute and Percent Change)

With regards to absolute data for HR, no interaction ($P = 1.00$), condition ($P = 0.95$), or time effect ($P = 0.11$) was noted, with values decreasing slightly following ingestion of the conditions. Absolute data are presented in Table 2. Regarding the percent change data for HR (Figure 1), no interaction effect ($P = 0.99$) was noted. However, a condition effect ($P = 0.02$) was noted, with C + G 50 mg higher than C and C + G 75 mg ($P < 0.05$). A time effect ($P = 0.0004$) was also noted, with 30, 60, 90, and 120 minutes lower than pre-ingestion ($P < 0.05$).

With regards to absolute data for SBP, no interaction effect ($P = 0.75$) was noted. However, a condition effect ($P = 0.0001$) was noted, with G 75 mg and C + G 75 mg greater than C, and C + G 75 mg greater than G 50 mg ($P < 0.05$). A time effect ($P < 0.0001$) was also noted, with 60, 90, and 120 minutes > 30 minutes and pre-ingestion ($P < 0.05$). Absolute data are presented in Table 3. Regarding the percent change data for SBP (Figure 2), no interaction effect ($P = 0.21$) was noted. However, a condition effect ($P < 0.0001$) was noted, with G 75 mg and C + G 75 mg greater than C; C + G 75 mg greater than G 50 mg and C + G 50 mg; and G 75 mg greater than G 50 mg ($P < 0.05$). A time effect ($P < 0.0001$) was also noted, with 60, 90, and 120 minutes greater than 30 minutes and pre-ingestion ($P < 0.05$).

With regards to absolute data for DBP, no interaction effect ($P = 1.00$) or condition effect ($P = 0.19$) was noted. However, a time effect ($P = 0.0004$) was noted, with 60, 90, and 120 minutes greater than pre-ingestion ($P < 0.05$). Absolute data are presented in Table 4. Regarding the percent change data for DBP (Figure 3), no interaction effect ($P = 1.00$) or condition effect ($P = 0.38$) was noted. However, a time effect ($P < 0.0001$) was noted, with 60, 90, and 120 minutes greater than pre-ingestion ($P < 0.05$).

With regards to absolute data for RPP, no interaction ($P = 1.00$) or time effect ($P = 0.26$) was noted. However, a condition effect ($P = 0.04$) was noted, with C + G 75 mg greater than C ($P < 0.05$). Absolute data are presented in Table 5. Regarding the percent change data for RPP (Figure 4), no interaction effect ($P = 0.65$) was noted. However, a condition effect ($P < 0.0001$) was noted, with G 75 mg, C + G 50 mg, and C + G 75 mg greater than C; C + G 50 mg greater than G 50 mg ($P < 0.05$). A time effect ($P = 0.006$) was also noted, with 120 minutes greater than 30 minutes and pre-ingestion; 60 minutes was greater than 30 minutes ($P < 0.05$).

The correlation analysis indicated a significant negative correlation between body weight and percentage change in SBP for G 50 mg ($r = -0.85$; $P = 0.002$). No other significant correlations were noted for any other condition with regards

Table 2. Heart Rate (bpm) Pre- and Post-Ingestion of Caffeine and Geranamine Alone or in Combination

Time	Caffeine 250 mg	Geranamine 50 mg	Geranamine 75 mg	Caffeine 250 mg + Geranamine 50 mg	Caffeine 250 mg + Geranamine 75 mg
Pre-ingestion	60 ± 3	60 ± 2	59 ± 3	59 ± 3	62 ± 2
30 min	56 ± 3	56 ± 2	56 ± 2	58 ± 2	58 ± 2
60 min	55 ± 3	57 ± 2	56 ± 3	57 ± 2	56 ± 3
90 min	55 ± 3	56 ± 3	56 ± 3	56 ± 2	56 ± 3
120 min	56 ± 3	57 ± 3	57 ± 2	58 ± 2	57 ± 3

Data are mean ± standard error of mean.
No condition × time interaction effect ($P = 1.00$).
No condition effect ($P = 0.95$).
No time effect ($P = 0.11$).

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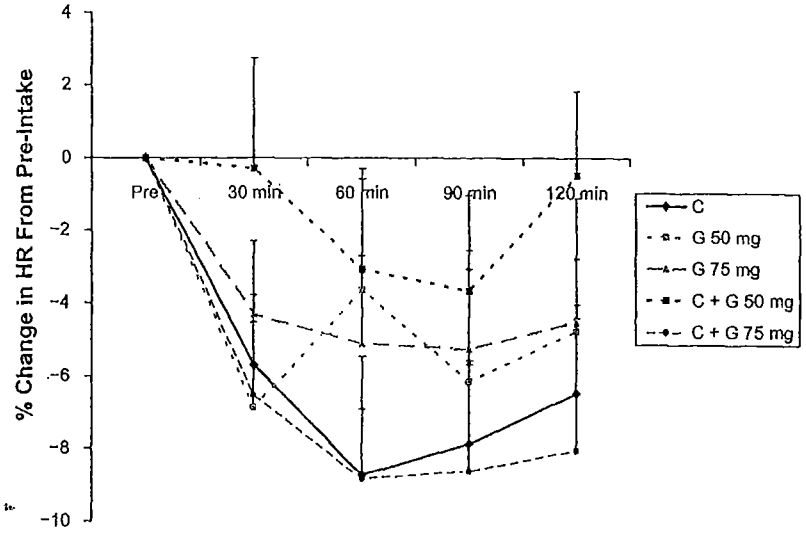
Figure 1. Percent change in heart rate from pre-intake of caffeine and geranamine alone or in combination.



Data are mean ± standard error of mean.
No condition × time interaction effect ($P = 0.99$).
Condition effect ($P = 0.02$); caffeine 250 mg + geranamine 50 mg different than caffeine 250 mg and caffeine 250 mg + geranamine 75 mg ($P = 0.05$).
Time effect ($P = 0.0004$); 30 min, 60 min, 90 min, 120 min different than pre-ingestion ($P < 0.05$).
Abbreviations: C, caffeine 250 mg; G 50 mg, geranamine 50 mg; G 75 mg, geranamine 75 mg; C + G 50 mg, caffeine 250 mg + geranamine 50 mg; C + G 75 mg, caffeine 250 mg + geranamine 75 mg.

to body weight or sex ($P > 0.05$). No significant correlations were noted for body weight or sex, for any condition, in relation to percentage change in DBP ($P > 0.05$).

Catecholamine Data

With regards to NE, no interaction ($P = 0.99$) or condition effect ($P = 0.99$) was noted. However, a time effect ($P = 0.01$) was noted, with 60 minutes lower than pre-ingestion and 120 minutes ($P < 0.05$). For EPI, no interaction ($P = 0.59$), condition ($P = 0.20$), or time effect ($P = 0.06$) was noted, but values generally increased at 60 minutes (6%) and 120 minutes (23%) compared with pre-ingestion. Data are presented in Table 6.

Discussion

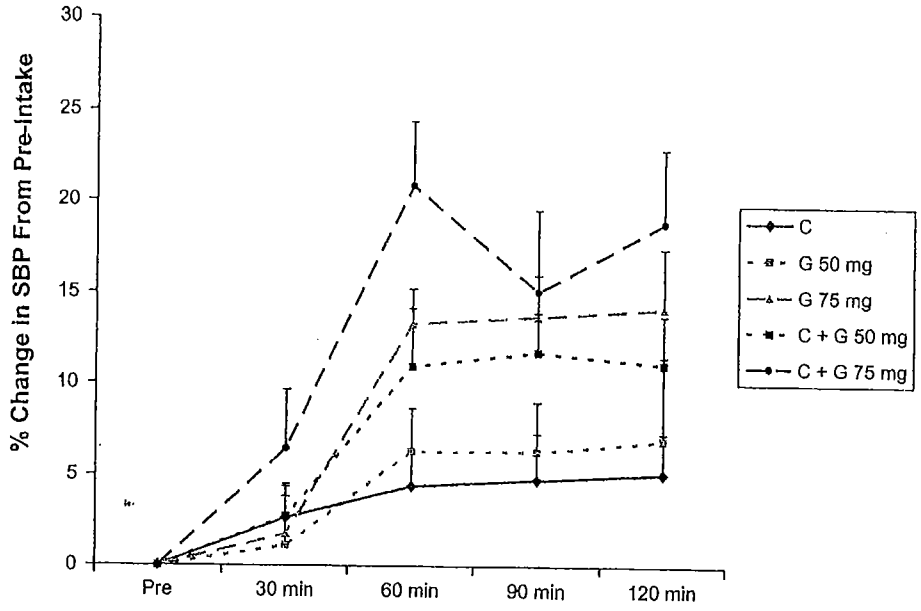
Data from the present study indicate that oral geranamine intake by healthy men and women results in an increase in SBP, DBP, and RPP without impacting HR. This is supported by observed changes in both absolute (SBP, DBP) and percent change values (SBP, DBP, RPP). The addition of caffeine to geranamine (at a dosage of 50 mg) results in an additive effect on the percent increase in RPP (Figure 4), but does not increase the response for any other variable, assuming the same dosage, in a statistically significant manner. The greatest increase in SBP and DBP is observed between 60 minutes and 90 minutes post-ingestion, in particular with the C + G 75 mg condition. These changes cannot be

Table 3. Systolic Blood Pressure (mm Hg) Pre- and Post-Ingestion of Caffeine and Geranamine Alone or in Combination

Time[b]	Caffeine 250 mg	Geranamine 50 mg	Geranamine 75 mg[a]	Caffeine 250 mg + Geranamine 50 mg	Caffeine 250 mg + Geranamine 75 mg[a]
Pre-ingestion	117 ± 3	121 ± 4	118 ± 4	119 ± 4	119 ± 3
30 min	120 ± 4	122 ± 4	120 ± 3	122 ± 4	126 ± 4
60 min	122 ± 4	128 ± 4	133 ± 5	131 ± 4	143 ± 5
90 min	122 ± 3	128 ± 4	134 ± 4	133 ± 4	136 ± 4
120 min	123 ± 5	128 ± 4	132 ± 4	132 ± 4	141 ± 5

Data are mean ± standard error of mean.
No condition × time interaction effect ($P = 0.75$).
[a]Condition effect ($P = 0.0001$); geranamine 75 mg and caffeine 250 mg + geranamine 75 mg different than caffeine 250 mg; caffeine 250 mg + geranamine 75 mg different geranamine 50 mg ($P < 0.05$).
[b]Time effect ($P < 0.0001$); 60, 90, and 120 minutes different than 30 minutes and pre-ingestion ($P < 0.05$).

Figure 2. Percent change in systolic blood pressure from pre-intake of caffeine and geranamine alone or in combination.



Data are mean ± standard error of mean.
No condition × time interaction effect ($P = 0.21$).
Condition effect ($P < 0.0001$); geranamine 75 mg and caffeine 250 mg + geranamine 75 mg different than caffeine 250 mg; caffeine 250 mg + geranamine 75 mg different than geranamine 50 mg and caffeine 250 mg + geranamine 50 mg; geranamine 75 mg different than geranamine 50 mg ($P < 0.05$).
Time effect ($P < 0.0001$); 60, 90, 120 minutes different than 30 minutes and pre-ingestion ($P < 0.05$).
Abbreviations: C, caffeine 250 mg; G 50 mg, geranamine 50 mg; G 75 mg, geranamine 75 mg; C + G 50 mg, caffeine 250 mg + geranamine 50 mg; C + G 75 mg, caffeine 250 mg + geranamine 75 mg; SBP, systolic blood pressure.

explained by the increase in NE and EPI, as the caffeine and geranamine appear to drive this response directly, as opposed to indirectly by increasing NE and EPI. Finally, there does not appear to be any significant influence of sex or body weight on these changes, with a possible exception of body weight being negatively correlated to the change in SBP following ingestion of G 50 mg. To our knowledge, these are the first data in human subjects to describe the effects of oral geranamine intake on HR and blood pressure.

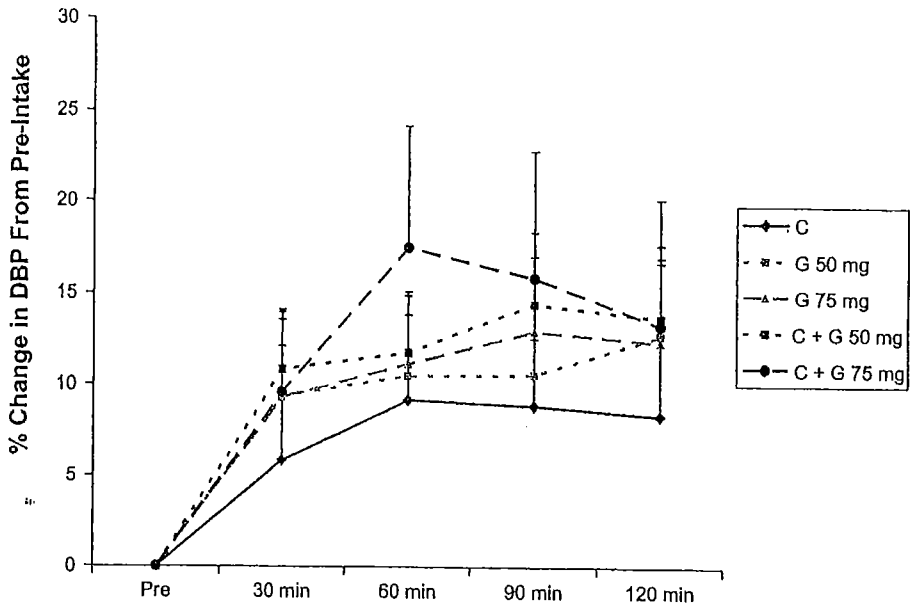
Other studies involving caffeine alone,[16,21,22] as well as caffeine combined with agents such as yohimbine and PEA[17] have noted similar findings for HR, SBP, and DBP as in the present study. Although we were somewhat surprised by a decrease in HR with all treatments (when expressed as a percent change from pre-ingestion), some prior studies involving caffeine have noted similar findings.[23,24] Based on the lack of an observed increase in HR, it is likely that the explanation for the increase in blood pressure involves an increase in total peripheral resistance and/or an increase in stroke volume. Indeed, the vasoconstriction effect of geranamine is supported by Eli Lilly's trademark application for Forthane™, which states "vasoconstrictor preparation."

Table 4. Diastolic Blood Pressure (mm Hg) Pre- and Post-Ingestion of Caffeine and Geranamine Alone or in Combination

Time[a]	Caffeine 250 mg	Geranamine 50 mg	Geranamine 75 mg	Caffeine 250 mg + Geranamine 50 mg	Caffeine 250 mg + Geranamine 75 mg
Pre-ingestion	70 ± 3	69 ± 4	70 ± 4	68 ± 3	71 ± 3
30 min	74 ± 4	75 ± 4	76 ± 2	75 ± 3	77 ± 3
60 min	76 ± 3	76 ± 4	77 ± 3	76 ± 3	83 ± 3
90 min	76 ± 3	76 ± 4	79 ± 3	78 ± 3	71 ± 3
120 min	75 ± 3	77 ± 4	78 ± 3	77 ± 3	80 ± 4

Data are mean ± standard error of mean.
No condition × time interaction effect ($P = 1.00$).
No condition effect ($P = 0.19$).
[a]Time effect ($P = 0.0004$); 60, 90, and 120 minutes different than pre-ingestion ($P < 0.05$).

Figure 3. Percent change in diastolic blood pressure from pre-intake of caffeine and geranamine alone or in combination.



Data are mean ± standard error of mean.
No condition × time interaction effect ($P = 1.00$).
No condition effect ($P = 0.38$).
Time effect ($P < 0.0001$); 30, 60, 90, and 120 min different than Pre ($P < 0.05$).
Abbreviations: C, caffeine 250 mg; G 50 mg, geranamine 50 mg; G 75 mg, geranamine 75 mg; C + G 50 mg, caffeine 250 mg + geranamine 50 mg; C + G 75 mg, caffeine 250 mg + geranamine 75 mg; DBP, diastolic blood pressure.

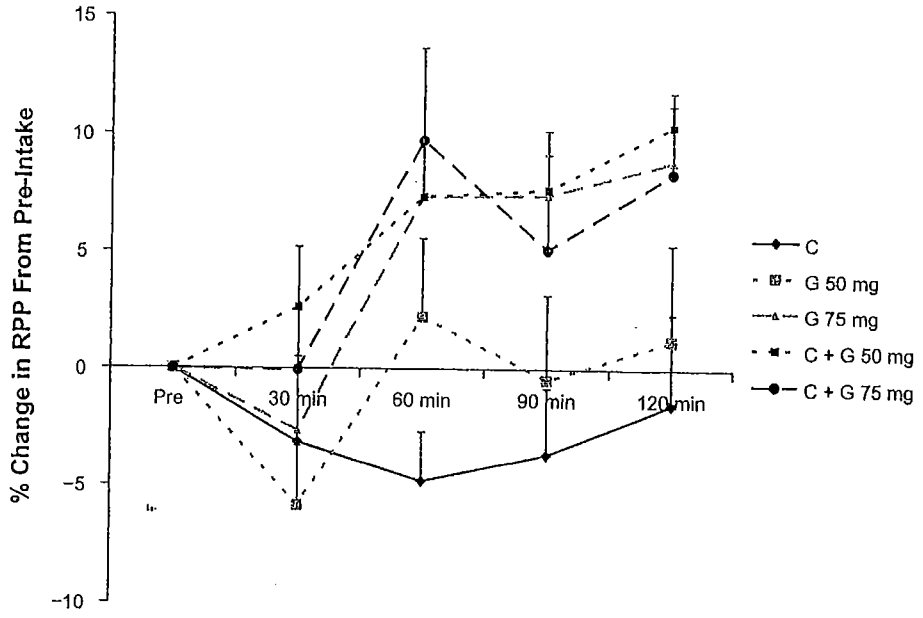
It is well accepted that an increase in catecholamine hormones may promote an increase in both vasoconstriction and cardiac contractility (promoting the above-mentioned effects). However, the greatest increase in NE and EPI was noted with caffeine intake alone, which was also associated with the smallest increase in blood pressure. Therefore, other mechanisms aside from circulating catecholamines must be responsible for our findings. This is likely mediated by a direct effect of geranamine on SBP as opposed to an indirect effect mediated by increasing NE and EPI. Although it has been reported that geranamine is less active than EPI in elevating blood pressure in animals,[3] the comparative effects in human subjects is unknown. Aside from a direct effect of geranamine, it is possible that an increase in sympathetic nervous system activity could be observed, irrespective of circulating catecholamines. Finally, it is also possible that catecholamine secretion at times other than 60 and 120 minutes post-ingestion could have influenced our findings. For example, we noted the peak increase in both SBP and DBP between 60 and 90 minutes post-ingestion, but we also measured blood NE and EPI at 60 minutes post-ingestion. Perhaps NE and EPI were peaking at times prior to the 60-minute post-ingestion measure, and hence mediating the increase in SBP and DBP observed at the 60-minute post-ingestion time. Considering our limited blood sampling, this possibility cannot be ruled out. Future studies attempt-

Table 5. Rate Pressure Product Pre- and Post-Ingestion of Caffeine and Geranamine Alone or in Combination

Time	Caffeine 250 mg	Geranamine 50 mg	Geranamine 75 mg	Caffeine 250 mg + Geranamine 50 mg	Caffeine 250 mg + Geranamine 75 mg[a]
Pre-ingestion	7015 ± 376	7294 ± 459	6996 ± 376	6993 ± 443	7356 ± 380
30 min	6817 ± 472	6824 ± 406	6776 ± 338	7122 ± 433	7271 ± 375
60 min	6669 ± 381	7348 ± 349	7539 ± 489	7427 ± 401	7995 ± 338
90 min	6770 ± 426	7157 ± 355	7492 ± 385	7420 ± 355	7595 ± 254
120 min	6897 ± 443	7247 ± 331	7567 ± 366	7688 ± 496	7929 ± 387

Data are mean ± standard error of mean.
No condition × time interaction effect ($P = 1.00$).
[a]Condition effect ($P = 0.04$); caffeine 250 mg + geranamine 75 mg different than caffeine 250 mg ($P < 0.05$).
No time effect ($P = 0.26$).

Figure 4. Percent change in rate pressure product from pre-intake of caffeine and geranamine alone or in combination.



Data are mean ± standard error of mean.
No condition × time interaction effect ($P = 0.65$).
Condition effect ($P < 0.0001$); geranamine 75 mg, caffeine 250 mg + geranamine 50 mg, and caffeine 250 mg + geranamine 75 mg is different than caffeine 250 mg; caffeine 250 mg + geranamine 50 mg different than geranamine 50 mg ($P < 0.05$).
Time effect ($P = 0.006$); 120 min different than 30 min and Pre; 60 min different than 30 min ($P < 0.05$).
Abbreviations: C, caffeine 250 mg; G 50 mg, geranamine 50 mg; G 75 mg, geranamine 75 mg; C + G 50 mg, caffeine 250 mg + geranamine 50 mg; C + G 75 mg, caffeine 250 mg + geranamine 75 mg; RPP, rate pressure product.

ing to describe the mechanistic effects of geranamine in relation to blood pressure may consider multiple and frequent sampling times for a more complete analysis of circulating catecholamines.

In the lay press, it has been suggested that geranamine may have similar functional properties as ephedrine. Ephedrine has been studied previously and noted to exhibit mixed results in terms of HR and blood pressure, with most work indicating a moderate increase in these variables.[25–28] For example, a study by Haller et al[27] noted an increase in SBP of 14 mm Hg at 90 minutes post-ingestion with a combination of caffeine (200 mg) and ephedrine alkoloids (20 mg).[27] Interestingly, the HR peaked by 15 beats per minute above baseline at 6 hours post-ingestion. This is an important consideration in relation to the present data, as we ceased measurement at 2 hours post-ingestion. Although it is possible that HR,

Table 6. Plasma NE and EPI Pre- and Post-Ingestion of Caffeine and Geranamine Alone or in Combination

Time	Caffeine 250 mg	Geranamine 50 mg	Geranamine 75 mg	Caffeine 250 mg + Geranamine 50 mg	Caffeine 250 mg + Geranamine 75 mg
NE ($pg \cdot mL^{-1}$)					
Pre	306 ± 34	339 ± 47	328 ± 46	327 ± 46	321 ± 41
60 min[a]	254 ± 32	267 ± 32	275 ± 49	272 ± 37	293 ± 41
120 min	383 ± 44	335 ± 40	340 ± 49	338 ± 38	340 ± 27
EPI ($pg \cdot mL^{-1}$)					
Pre	51 ± 8	72 ± 10	70 ± 8	47 ± 9	71 ± 8
60 min	56 ± 8	63 ± 12	73 ± 9	69 ± 10	71 ± 9
120 min	84 ± 7	80 ± 12	83 ± 11	60 ± 10	77 ± 10

Data are mean ± standard error of mean.
No condition × time interaction effect for NE ($P = 0.99$) or EPI ($P = 0.59$).
No condition effect for NE ($P = 0.99$) or EPI ($P = 0.20$).
[a]Time effect for NE ($P = 0.01$); 60 min lower than pre-ingestion and 120 min ($P < 0.05$).
No time effect for EPI ($P = 0.06$).
Abbreviations: EPI, epinephrine; NE, norepinephrine.

SBP, or DBP could have been increased at times beyond our 2-hour post-ingestion period, percent change data presented in Figures 1 to 3 indicate that values (for the most part) were stable or returned toward baseline by 120 minutes. Indeed, future work is needed to determine the longer-term effects of geranamine alone and in combination with caffeine on HR and blood pressure. Investigators interested in this area of research are encouraged to pursue additional studies, as very few data are currently available pertaining to this ingredient, despite its widespread use.

Of potential concern in relation to our findings for geranamine is the increase in blood pressure in response to treatment, in particular at a dosage of 75 mg. Even when combined with 250 mg of caffeine, a dosage of 50 mg of geranamine did not result in as significant an increase in SBP, and a similar increase in DBP, as compared with 75 mg of geranamine alone (when presented as percent change from pre-ingestion; Figures 2, 3). This finding for geranamine was rather uniform, although the magnitude of effect varied across subjects, which is typical for many nutrients and drugs. Although acute ingestion of geranamine does appear to increase blood pressure, it should be made clear that data from this study do not provide us with the needed evidence to comment on the effect of chronic geranamine ingestion with regards to blood pressure elevation. That is, at present there is no evidence to indicate that routine use of this agent results in hypertension. This is underscored by the reported tachyphylaxis for this agent.[3] Despite this, based on the acute elevation in blood pressure observed following intake of geranamine by healthy men and women, it would likely be prudent for individuals with known hypertension to avoid use of this agent. Additional work is needed to determine the potential impact of regular geranamine ingestion on HR and blood pressure over time, in addition to the clinical relevance that any noted changes in these variables might have.

Conclusion

We report for the first time that acute oral geranamine intake by healthy men and women results in a significant increase in blood pressure, without impacting HR. The effect appears dose dependent, in particular for SBP, with a greater increase at 75 mg compared with 50 mg. The addition of caffeine to geranamine (at a dosage of 50 mg) increases the percent change from pre-ingestion in RPP, but does not influence other variables in a statistically significant manner. The changes in HR and blood pressure cannot be explained by circulating NE and EPI. Future studies are needed to determine what, if any, change in resting HR and blood pressure may be noted with chronic ingestion of geranamine.

Acknowledgments

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Conflict of Interest Statement

Richard J. Bloomer, PhD discloses conflicts of interest with Advanced Oral Technologies, Kaneka Nutrients, Formulife, and USPLabs. Innocence C. Harvey, BS, Tyler M. Farney, MS, Zach W. Bell, BS, and Robert E. Canale, MS disclose no conflicts of interest.

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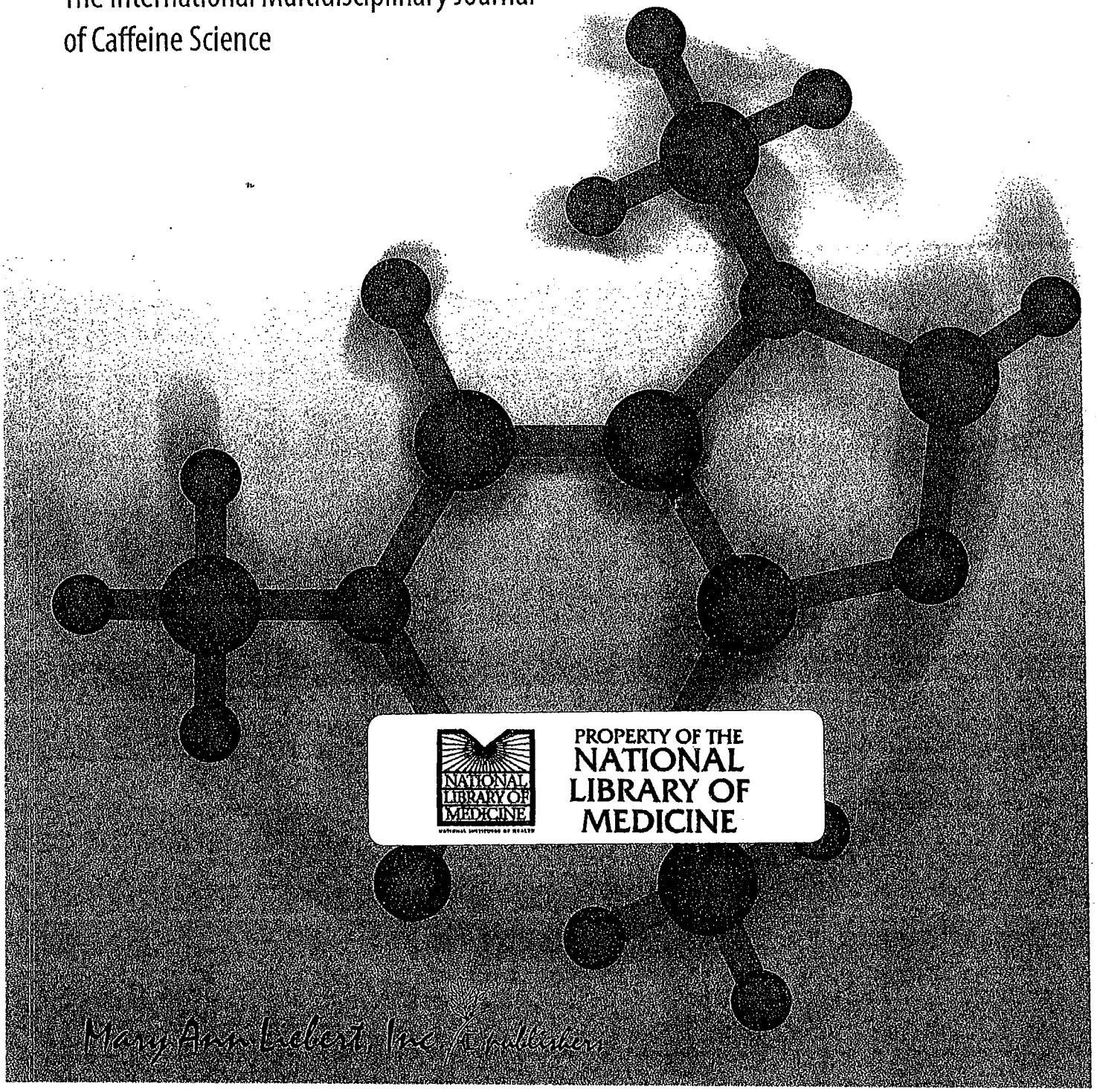

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Effect of Caffeine and 1,3-Dimethylamylamine on Exercise Performance and Blood Markers of Lipolysis and Oxidative Stress in Trained Men and Women

Richard J. Bloomer, Cameron G. McCarthy, Tyler M. Farney, and Innocence C. Harvey

Background: Caffeine is one of the most widely used ergogenic aids worldwide. Recently, caffeine has been combined with 1,3-dimethylamylamine (1,3-D) in an attempt to improve exercise performance and related variables. We investigated the effect of caffeine and 1,3-D alone and in combination on exercise performance and blood markers of lipolysis and oxidative stress.
Methods: Twelve exercise-trained subjects ingested placebo, caffeine ($4\ mg \cdot kg^{-1}$), 1,3-D ($1\ mg \cdot kg^{-1}$), or caffeine + 1,3-D, 60 minutes before completing a 10 km run. Blood was collected before intake, immediately pre-exercise, and at 5 and 30 minutes postexercise. Samples were analyzed for glycerol, free fatty acids (FFAs), malondialdehyde, nitrate/nitrite, and trolox equivalent antioxidant capacity (TEAC).
Results: Run time (minutes) was not different for placebo (52.55 ± 1.96), caffeine (52.00 ± 1.88), 1,3-D (52.02 ± 1.86), or caffeine + 1,3-D (52.46 ± 1.94) ($p > 0.05$). Glycerol and FFA were higher 5 and 30 minutes postexercise compared with pretreatment and pre-exercise ($p < 0.05$). A condition effect was noted for glycerol ($p = 0.01$), with higher values for 1,3-D compared with caffeine + 1,3-D ($p < 0.05$). A condition effect was noted for TEAC ($p = 0.0001$), with higher values for placebo compared with caffeine and caffeine + 1,3-D, and higher values for 1,3-D compared with caffeine ($p < 0.05$). No other effects were noted for any measured variable ($p > 0.05$).
Conclusion: We report for the first time that caffeine + 1,3D does not improve exercise performance as measured by run time. Isolated ingestion of 1,3-D results in the greatest increase in postexercise glycerol and FFA. Caffeine or 1,3-D alone or in combination does not differently affect oxidative stress biomarkers.

Introduction

Caffeine is arguably one of the most widely used ergogenic aids in the world[1]—included within hundreds of different encapsulated, pilled, and powdered dietary supplements. One recent addition to the ergogenic supplement category is a component of the Pelargonium graveolens plant (1,3-dimethylamylamine [1,3-D]),[2] which has anecdotally been noted to possess a powerful stimulatory effect. The combination of caffeine and 1,3-D is becoming increasingly popular among athletes due to the potential for performance enhancement, as well as related effects. Specifically, caffeine has been described as a lipolytic agent,[3–5] allowing for the release of free fatty acids (FFAs) from storage depots. However, data pertaining to the lipolytic effects of caffeine are mixed, with some studies reporting no increase in lipolysis after caffeine ingestion[6,7] or a derivative of caffeine.[8] The literature pertaining to the use of 1,3-D is scant; however, this agent is a simple aliphatic amine that functions as a norepinephrine reuptake inhibitor and/or norepinephrine releasing agent.[9] Based on these effects, 1,3-D may lead to enhanced hormone sensitive lipase activity, ultimately allowing for the release of FFA to undergo oxidation for energy production purposes.[10]

Apart from the potential lipolytic effects of these agents, both caffeine and 1,3-D appear to enhance focus and attention during exercise. Although scientific evidence is available to support this effect for caffeine,[11,12] only anecdotal reports are available for 1,3-D. Considering what has been just stated, the combination of these two agents may aid exercise performance due to a potential lipolytic effect, thus allowing for increased FFA to be available as a fuel source during moderate duration exercise, potentially sparing muscle glycogen, while also enhancing focus during training.

Beyond what has been just stated, evidence from nonexercising humans indicates that caffeine may provide antioxidant benefits,[13,14] thus potentially attenuating oxidative stress. This antioxidant effect has also been noted for 1,3-D.[15] The improvement in antioxidant status may be attractive to exercise enthusiasts, as increased oxidative stress is often noted in response to strenuous physical work.[16] It is possible that the use of caffeine and 1,3-D alone or in combination may serve to minimize the oxidative stress resulting from an

Cardiorespiratory/Metabolic Laboratory, Department of Health and Sport Sciences, University of Memphis, Memphis, Tennessee.

acute bout of strenuous exercise. To date, no studies have determined this.

Although both 1,3-D and caffeine are known to acutely elevate blood pressure,[17] caffeine has also been noted to induce vasorelaxation by stimulating the production of nitric oxide.[18] Collectively considering the above, we investigated the effect of caffeine and 1,3-D on exercise performance and blood markers of lipolysis and oxidative stress in a sample of exercise-trained men and women. We also measured plasma nitrate/nitrite (NOx) and hemodynamic variables before and after treatment with these agents.

Materials and Methods

Subjects

Young and healthy, exercise-trained men ($n=6$) and women ($n=6$) participated in this investigation. All subjects completed a medical history and physical activity questionnaire to determine eligibility for participation. No subject was a smoker or had diagnosed cardiovascular (e.g., hypertension) or metabolic disease. All subjects were regular runners. Three subjects reported using caffeine daily, through consumption of coffee or espresso ($n=2$), or green tea ($n=1$). The mean daily caffeine intake for these subjects was 230 mg·day^{-1}. Three subjects reported using coffee infrequently throughout the week. Subjects' heart rate and blood pressure, height, weight, waist and hip circumference, and skinfold thickness (seven site) for estimation of body fat percentage were measured and used to describe the subjects. These descriptive characteristics, including exercise training history, are presented in Table 1. After screening procedures, the subjects performed a 3 km familiarization run on an outdoor track—that used for the experimental test days. All experimental procedures were performed in accordance with the Helsinki Declaration. The University of Memphis Human Subjects Committee approved all experimental procedures (H10-49), and subjects provided verbal and written consent before participating in this study.

Testing

All testing procedures described next were identical for all four test days. Subjects reported to the laboratory in a 10 hours fasted state, and all testing was completed in the morning hours (0500–0900). The time of testing was matched for subjects for all four conditions. Subjects were instructed not to exercise for the 24 hours before each test day. On arrival to the laboratory, the subjects were asked to void and then rested quietly for 10 minutes in a seated position. After this, the subjects' heart rate (HR: via radial artery palpation for 60 seconds by two trained technicians) and systolic (SBP) and diastolic (DBP) blood pressure (via auscultation using a dual earpiece stethoscope) were measured. Rate pressure product (RPP) was calculated as an indication of myocardial work by using the equation: HR×SBP. A blood sample was then collected. Subjects were then provided their assigned condition and ingested this in the presence of an investigator. After the ingestion of the assigned condition, the subjects rested for 60 minutes and then began the exercise bout. Before starting the exercise bout, HR and blood pressure were measured, and a blood sample was collected. This same collection (HR, blood pressure, and blood) was repeated at 5 and 30 minutes postexercise. Subjects consumed no food during the entire testing period; however, water was allowed *ad libitum* and matched for subjects on the days of testing.

The exercise bout consisted of a 10 km run on an outdoor track. For all bouts, subjects were encouraged to complete the run as quickly as possible. HR was monitored by using a HR monitor. The Borg (6–20) scale of exertion was used to allow subjects to indicate their level of perceived work. Subjects were also asked to rate their overall mood/vigor by using a 0–10 point scale. HR, perceived exertion, and mood/vigor were recorded at the end of each 2 km period. At the end of exercise, run time was recorded, and the subjects returned to the lab for the postexercise measures. It should be noted that the walk from the track to the lab is estimated to be <150 feet. Test days were separated by 1 week. The environmental conditions for each test day were recorded and noted to be very good for running. For example, starting run temperatures ranged from 44°F to 68°F, with mostly clear skies on all test days and wind noted only on three of the 18 days on which the subjects ran.

Table 1. Characteristics of 12 Exercise-Trained Subjects

Variable	*Value*
Age (years)	21.9±2.9
Height (cm)	173.2±10.7
Weight (kg)	67.6±11.9
Body mass index (kg·m^{-2})	22.4±2.5
Body fat (%)	15.6±7.8
Waist (cm)	72.7±6.8
Hip (cm)	96.8±4.8
Waist:Hip	0.75±0.04
Years of anerobic exercise training	4.5±2.8
Hours per week of anerobic exercise	3.0±1.6
Years of aerobic exercise training	5.2±3.7
Hours per week of aerobic exercise	6.3±4.3

Data are mean±standard deviation.

Conditions

Conditions were received in a random order by using a double-blind design: placebo (30 g of carbohydrate); caffeine (30 g of carbohydrate+caffeine at 4 mg·kg body mass^{-1}); 1,3-D (30 g of carbohydrate+ 1,3-D at 1 mg·kg body mass^{-1}); or caffeine+ 1,3-D (30 g of carbohydrate+ caffeine at 4 mg·kg body mass^{-1} and 1,3-D at 1 mg·kg body mass^{-1}). The dosage of caffeine used in the current design was based on previous studies using caffeine for an ergogenic benefit—in particular, one study using caffeine at a dosage of 3 mg·kg body mass^{-1} and noting an improvement in 8 km run performance.[19] However, it should be noted that many studies have used caffeine at a dosage >4 mg·kg body mass^{-1}.[20–23] Our use of a relatively low dosage of caffeine was based on our inclusion of 1,3-D in the current design, and our concern over excessive stimulation with higher dosing. The dosage of 1,3-D was based on anecdotal reports of individuals using this ingredient, coupled with a review of nutritional panels of dietary supplements containing this ingredient.

The 1,3-D (1,3-dimethylamylamine HCL) was purchased from Waseta International (Shanghia, China), and the caffeine (caffeine anhydrous) was purchased from Hi Tech

TABLE 2. DIETARY DATA OF EXERCISE-TRAINED SUBJECTS RECEIVING PLACEBO, CAFFEINE, 1,3-DIMETHYLAMYLAMINE, OR CAFFEINE+ 1,3-DIMETHYLAMYLAMINE

Variable	*Placebo*	*Caffeine*	*1,3-Dimethylamylamine*	*Caffeine+ 1,3-dimethylamylamine*
Kilocalories	2358±209	2083±212	2294±247	2070±238
Protein (g)	104±14	100±13	109±17	97±17
Carbohydrate (g)	329±38	331±44	320±45	283±42
Fat (g)	77±8	74±11	69±10	66±10
Vitamin C (mg)	90±31	90±28	96±25	81±21
Vitamin E (mg)	7±4	12±5	9±3	7±2
Vitamin A (RE)	417±146	551±164	643±191	463±135

Data are mean±SEM. No statistical difference was noted between conditions in kilocalories ($p=0.94$), protein ($p=0.76$), carbohydrate ($p=0.91$), fat ($p=0.44$), vitamin C ($p=0.99$), vitamin E ($p=0.77$), or vitamin A ($p=0.92$).
RE, retinol equivalents; SEM, standard error of the mean.

Pharmaceuticals, Inc. (Norcross, GA). Certificates of analysis for each ingredient indicated purity. All conditions were mixed into 500 mL of water and, the beverage was fruit-punch flavored. The carbohydrate source was maltodextrin, and our dosage of 30 g was selected to provide 120 kcal, an amount similar to what is provided within many commonly consumed sport drinks.

Blood collection and biochemistry

A total of four venous blood samples were taken from subjects' forearm veins via needle and Vacutainer®. Blood for collection of serum was allowed to clot for 30 minutes at room temperature and then processed in a refrigerated centrifuge (4°C for 15 minutes at 1500*g*). Blood for collection of plasma was immediately processed in a refrigerated centrifuge (4°C for 15 minutes at 1500*g*). Samples were stored in multiple aliquots at −70°C. Glycerol was analyzed in plasma by using the Free Glycerol Determination Kit (FG0100) and Glycerol Standard (G7793), following the manufacturer's instructions (Sigma Aldrich). FFAs were analyzed in plasma by using the Free Fatty Acid Quantification Kit (K612-100), following the manufacturer's instructions (BioVision). As a measure of lipid peroxidation, malondialdehyde (MDA) was analyzed in plasma by following the procedures of Jentzsch *et al.* using.[24] reagents purchased from Northwest Life Science Specialties (Vancouver, WA). As a surrogate measure of nitric oxide, NOx was analyzed in plasma by using a commercially available colorimetric assay kit (Cayman Chemical, Ann Arbor, MI) according to the procedures provided by the manufacturer. Antioxidant capacity was analyzed in serum by using the trolox equivalent antioxidant capacity (TEAC) assay using procedures outlined by the reagent provider (Sigma Chemical, St. Louis, MO). All samples were analyzed on first thaw.

Dietary records

All subjects were instructed to maintain their normal diet during the study period and to record all food and drink consumed during the 24 hours before each test day. Records were reviewed with each subject for accuracy and then analyzed using Food Processor SQL, version 9.9 (ESHA Research, Salem, OR).

Statistical analysis

Exercise performance and dietary data were analyzed by using an analysis of variance (ANOVA). All other data were analyzed by using a 4 (condition)×4 (time) ANOVA. Tukey *post hoc* tests were performed when necessary. Statistical significance was set at $p \leq 0.05$. The analyses were done using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC). Data are presented as mean±standard error of the mean, except for subject characteristics that are presented as mean±standard deviation.

Results

All subjects successfully completed all test days. The conditions were generally well-tolerated; however, the combination of caffeine+ 1,3-D resulted in extreme feelings of euphoria in many subjects, some of whom claimed that their exercise performance may have been better if not receiving the condition. Dietary data during the day before each test day were not different between conditions ($p>0.05$). Data are presented in Table 2.

Performance data

With regard to the performance data, run time ($p=0.99$), perceived exertion ($p=0.71$), mood/vigor ($p=0.41$), and HR ($p=0.91$) were not different between conditions. Data are presented in Table 3.

TABLE 3. RUN TIME, PERCEIVED EXERTION, MOOD/VIGOR, AND HEART RATE OF EXERCISE-TRAINED SUBJECTS RECEIVING PLACEBO, CAFFEINE, 1,3-DIMETHYLAMYLAMINE, OR CAFFEINE+ 1,3-DIMETHYLAMYLAMINE

Variable	*Placebo*	*Caffeine*	*1,3-Dimethylamylamine*	*Caffeine+ 1,3-dimethylamylamine*
Run time (minutes)	52.55±1.96	52.00±1.88	52.02±1.86	52.46±1.94
Perceived exertion (6–20 scale)	13.70±0.47	14.17±0.36	13.93±0.65	14.46±0.40
Mood/vigor (0–10 scale)	5.02±0.31	5.10±0.41	5.57±0.50	4.58±0.39
Heart rate (bpm)	180.61±3.23	178.53±3.94	181.83±2.84	179.43±3.62

Data are mean±SEM. No statistical difference was noted between conditions in run time ($p=0.99$), perceived exertion ($p=0.71$), mood/vigor ($p=0.41$), or heart rate ($p=0.91$). Perceived exertion, mood/vigor, and heart rate were recorded every 2 km. Data were averaged across all times for each subject, and mean data are presented in the table.

Biochemical data

With regard to the biochemical data, results were as follows. For glycerol, a condition effect was noted ($p=0.01$), with 1,3-D greater than caffeine+ 1,3-D ($p<0.05$). A time effect was noted ($p<0.0001$), with 5 minutes postexercise and 30 minutes postexercise greater than pretreatment and pre-exercise; 5 minutes postexercise >30 minutes postexercise ($p<0.05$). No condition×time interaction was noted ($p=0.23$); however, values were highest for 1,3-D and lowest for caffeine+ 1,3-D at the 5 and 30 minutes postexercise times. For FFA, no condition effect was noted ($p=0.06$). A time effect was noted ($p<0.0001$), with 5 minutes postexercise and 30 minutes postexercise greater than pretreatment and pre-exercise; pretreatment greater than pre-exercise; and 5 minutes postexercise >30 minutes postexercise ($p<0.05$). No condition×time interaction was noted ($p=0.14$); however, values were highest for 1,3-D at the 5 and 30 minutes postexercise times. Data for glycerol (A) and FFA (B) are presented in Figure 1.

For MDA, no condition ($p=0.84$), time ($p=0.83$), or condition×time interaction effect was noted ($p=0.98$). For NOx, no condition ($p=0.16$), time ($p=0.91$), or condition×time interaction effect was noted ($p=0.99$). For TEAC, a condition effect was noted ($p=0.0001$), with placebo greater than caffeine and caffeine+ 1,3-D; 1,3-D greater than caffeine ($p<0.05$). No time ($p=0.10$) or condition×time interaction effect was noted ($p=0.96$). Data for MDA (A), NOx (B), and TEAC (C) are presented in Figure 2.

Hemodynamic data

With regard to the hemodynamic data, results were as follows. For HR, a condition effect was noted ($p=0.02$), with caffeine+ 1,3-D greater than 1,3-D ($p<0.05$). A time effect was noted ($p<0.0001$), with 5 minutes postexercise and 30 minutes postexercise greater than pretreatment and pre-exercise ($p<0.05$). No condition×time interaction was noted ($p=0.94$). For SBP, a condition effect was noted ($p<0.0001$), with caffeine and 1,3-D greater than placebo and caffeine+ 1,3-D ($p<0.05$). A time effect was noted ($p<0.0001$), with pre-exercise and 5 minutes postexercise greater than pretreatment and 30 minutes postexercise ($p<0.05$). A condition×time interaction was also noted ($p<0.0001$). For DBP, a condition effect was noted ($p=0.03$), with 1,3-D greater than caffeine+ 1,3-D ($p<0.05$). A time effect was noted ($p<0.0001$), with pre-exercise greater than all other times ($p<0.05$). No condition×time interaction was noted ($p=0.23$). For RPP, no condition effect was noted ($p=0.33$). A time effect was noted ($p<0.0001$), with pretreatment less than all other times; 5 minutes postexercise and 30 minutes postexercise greater than pre-exercise; and 5 minutes postexercise >30 minutes postexercise ($p<0.05$). No condition×time interaction was noted ($p=0.95$). Data are presented in Table 4.

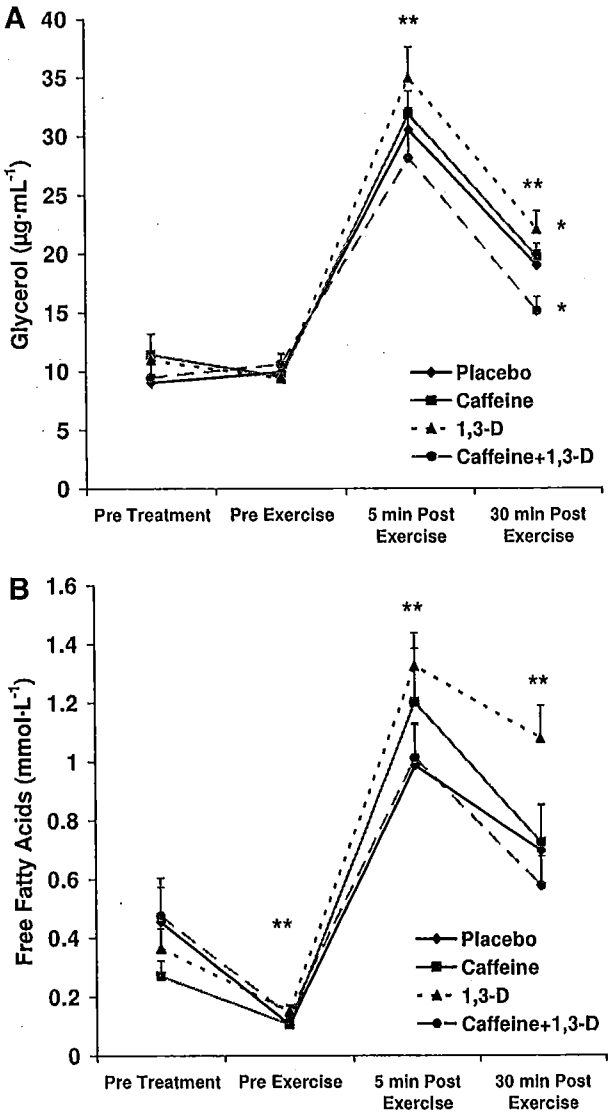
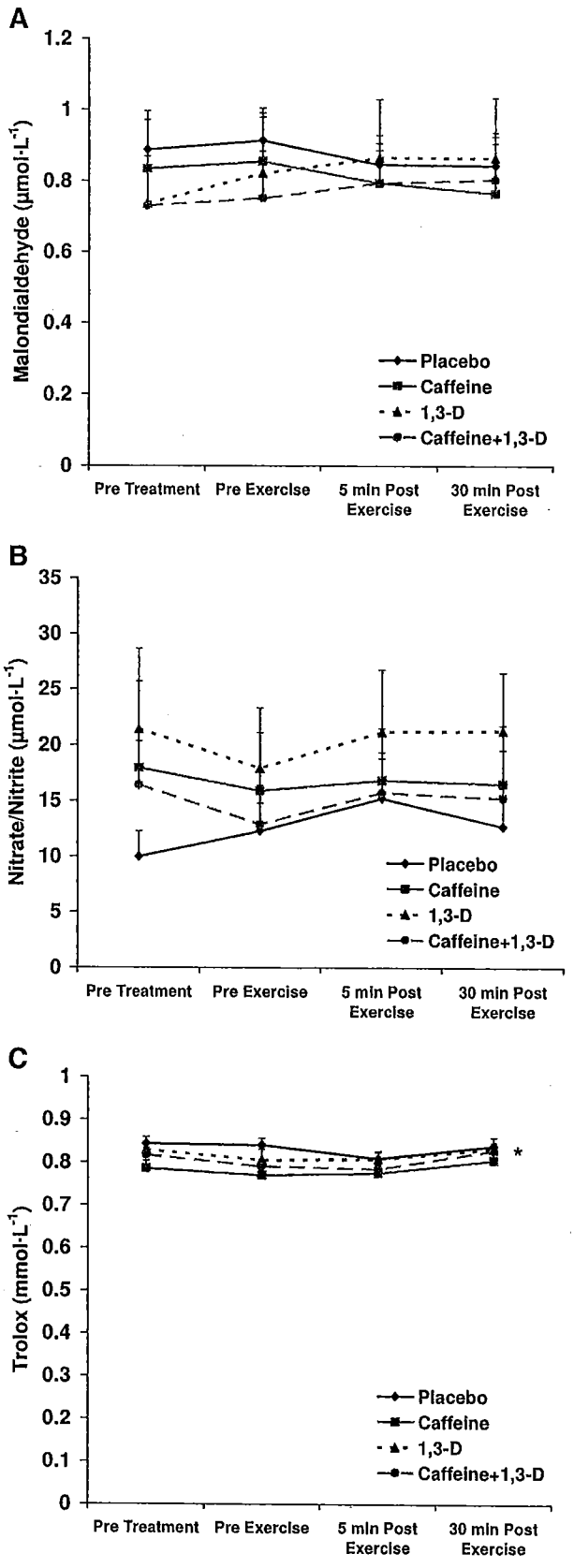


FIG. 1. Plasma glycerol (A) and free fatty acids (B) of exercise-trained subjects receiving placebo, caffeine, 1,3-dimethylamylamine, or caffeine+ 1,3-dimethylamylamine. Glycerol: *A condition effect was noted ($p=0.01$); 1,3-D>caffeine+ 1,3-D ($p<0.05$). **A time effect was noted ($p<0.0001$); 5 minutes postexercise and 30 minutes postexercise >pretreatment and pre-exercise; 5 minutes postexercise >30 minutes postexercise ($p<0.05$). No condition×time effect was noted ($p=0.23$). Free fatty acids: No condition effect was noted ($p=0.06$). **A time effect was noted ($p<0.0001$); 5 minutes postexercise and 30 minutes postexercise>pretreatment and pre-exercise; pretreatment>pre-exercise; and 5 minutes postexercise>30 minutes postexercise ($p<0.05$). No condition×time effect was noted ($p=0.14$).

Discussion

Data from the current investigation indicate that (1) ingestion of caffeine or 1,3-D alone or in combination does not improve exercise performance as measured by run time; (2) ingestion of 1,3-D results in the greatest increase in postexercise glycerol and FFA concentrations; (3) caffeine or 1,3-D alone or in combination does not differently effect oxidative stress biomarkers pre- or postexercise; and (4) caffeine and 1,3-D alone increase SBP. This is the first study to investigate the effect of 1,3-D alone and in combination with caffeine on exercise performance and related variables.



Our outcome of greatest interest in this investigation was that of exercise performance (run time). We noted no statistically significant effect of condition on run time, although times for both caffeine and 1,3-D were approximately 30 seconds lower than for placebo (Table 3). Although not statistically different, it is possible that such a subtle difference in run time might prove beneficial in competition. It is also possible that these ingredients may benefit performance during other exercise challenges (e.g., resistance exercise). Additional work is needed to determine this.

The combination of caffeine + 1,3-D did not result in as favorable of an outcome with regard to run time as compared with the two agents alone. We believe that the dosage of each agent was too high when delivered in combination, as many subjects reported feeling too euphoric before and during the exercise bout. Perhaps a lower dosage would have yielded more positive results. Our findings of no significant improvement in exercise performance for caffeine compared with placebo agree with several reports[25–30]; however, they are in conflict with others.[3,19–23,31–34] Multiple factors are likely involved in the discrepancy across studies, including caffeine dosage, the combination of caffeine with other dietary nutrients and stimulants, the inclusion of a pre-exercise meal, the type of exercise test being performed, the time of day of the exercise test relative to subjects' usual exercise time, where the exercise test is being performed, climatic temperature and conditions (assuming outdoors), and training status of subjects.

Beyond the measure of run time, no other performance-related measure was different between conditions (perceived exertion, mood/vigor, and HR). In support of subjects' comments, the combination of caffeine + 1,3-D yielded the highest perceived exertion and the lowest mood/vigor. Again, it is likely that the combined dosage of these two agents was simply too high, and subjects did not tolerate this condition well. Future work should seek to determine whether a lower dosage of each agent would result in a more favorable response—as has been frequently cited in anecdotal reports using a combination of these two agents. In agreement with the pre- and postexercise measure of HR, the exercise HR data were not different for caffeine or 1,3-D alone or in combination, as compared with placebo. These findings confirm that the treatment dosages used in the current study do not cause an exercise-induced exacerbation in HR.

As expected, we noted an increase in both glycerol and FFA in response to exercise (Fig. 1). These findings are well supported by the exercise literature, in particular with regard

FIG. 2. Plasma malondialdehyde (A), nitrate/nitrite (B), and trolox equivalent antioxidant capacity (C) of exercise-trained subjects receiving placebo, caffeine, 1,3-dimethylamylamine, or caffeine + 1,3-dimethylamylamine. Malondialdehyde: No condition effect was noted ($p = 0.84$). No time effect was noted ($p = 0.83$). No condition × time effect was noted ($p = 0.98$). nitrate/nitrite: No condition effect was noted ($p = 0.16$). No time effect was noted ($p = 0.91$). No condition × time effect was noted ($p = 0.99$). Trolox equivalent antioxidant capacity: *A condition effect was noted ($p = 0.0001$); placebo > caffeine and caffeine + 1,3-D; 1,3-D > caffeine ($p < 0.05$); No time effect was noted ($p = 0.10$). No condition × time effect was noted ($p = 0.96$).

TABLE 4. HEMODYNAMIC DATA OF EXERCISE-TRAINED SUBJECTS RECEIVING PLACEBO, CAFFEINE, 1,3-DIMETHYLAMYLAMINE, OR CAFFEINE + 1,3-DIMETHYLAMYLAMINE

Variable	*Heart rate (bpm)*	*Systolic blood pressure (mmHg)*	*Diastolic blood pressure (mmHg)*	*Rate pressure product*
Placebo Pretreatment	57±3	113±1	64±3	6394±289
Placebo Pre-exercise	60±2	121±3	67±3	7202±286
Placebo 5 minutes postexercise	109±3	126±3	64±2	13,711±505
Placebo 30 minutes postexercise	85±4	112±3	67±2	9543±532
Caffeine Pretreatment	59±2	113±2	65±2	6670±259
Caffeine Pre-exercise	59±2	140±3	75±5	8265±339
Caffeine 5 minutes postexercise	104±6	141±4	65±3	14,596±939
Caffeine 30 minutes postexercise	79±5	125±4	65±3	9913±696
1,3-D Pretreatment	55±2	113±3	66±2	6175±247
1,3-D Pre-exercise	53±2	150±5	81±3	8033±420
1,3-D 5 minutes postexercise	100±5	147±4	66±3	14,787±944
1,3-D 30 minutes postexercise	78±4	128±4	69±3	10,014±725
Caffeine + 1,3-D Pretreatment	58±3	117±3	63±2	6845±417
Caffeine + 1,3-D Pre-exercise	62±3	120±2	67±3	7351±340
Caffeine + 1,3-D 5 minutes postexercise	112±5	126±3	61±2	14,181±780
Caffeine + 1,3-D 30 minutes postexercise	83±3	111±3	69±3	9256±438

Data are mean±SEM. *Heart rate*: Condition: $p=0.02$; caffeine + 1,3-D > 1,3-D ($p<0.05$). Time: $p<0.0001$; 5 minutes postexercise and 30 minutes postexercise > pretreatment and pre-exercise ($p<0.05$). Condition × Time: $p=0.94$. *Systolic blood pressure*: Condition: $p<0.0001$; caffeine and 1,3-D > placebo and caffeine + 1,3-D ($p<0.05$). Time: $p<0.0001$; pre-exercise and 5 minutes postexercise > pre-treatment and 30 minutes postexercise ($p<0.05$). Condition × Time: $p<0.0001$. Diastolic blood pressure: Condition: $p=0.03$; 1,3-D > caffeine + 1,3-D ($p<0.05$). Time: $p<0.0001$; pre-exercise > all other times ($p<0.05$). Condition × Time: $p=0.23$. *Rate pressure product*: Condition: $p=0.33$. Time: $p<0.0001$; pretreatment < all other times; 5 minutes postexercise and 30 minutes postexercise > pre-exercise; 5 minutes postexercise > 30 minutes postexercise ($p<0.05$). Condition × Time: $p=0.95$.

1,3-D, 1,3-dimethylamylamine.

to aerobic exercise[23,32,35] However, we found no interaction effects for either glycerol or FFA, with a relatively similar response curve noted for all conditions. Some studies using caffeine as a lipolytic agent have noted increased lipolysis at rest[36,37] and with exercise.[23,31,32] However, others have not,[25,26,35,38,39] essentially corroborating our findings or little difference between the caffeine and placebo conditions either pre- or postexercise.

A condition effect was noted for glycerol, with 1,3-D exhibiting the greatest increase postexercise—statistically higher than the combination of caffeine + 1,3-D. The same general finding was noted for FFA, with 1,3-D demonstrating the highest overall values; however, the condition effect failed to reach significance ($p=0.06$). Interestingly, the combination of caffeine + 1,3-D demonstrated the lowest values for both glycerol and FFA, which somewhat helps explain the low-performance-related findings. Based on the assumed lipolytic properties of both caffeine and 1,3-D, we do not have a clear explanation as to why both glycerol and FFA concentrations were lowest in the combined condition—although this may be related to the hormonal response to treatment. For example, if a hormone such as insulin was elevated to a greater extent with the combined treatment, then this may help explain our findings for lower FFA and glycerol, as elevations in insulin[40] are known to suppress fatty acid release and oxidation. Of course, we have no evidence to support this hypothesis, in particular when considering that greater insulin release is not observed with caffeine intake alone.[41] Clearly, neither caffeine or 1,3-D alone resulted in an impairment in FFA and glycerol release (Fig. 1). Therefore, if our hypothesis just stated were confirmed, then there would need to be some synergy between the two treatments to promote this suppression in response.

The increase in glycerol and FFA with 1,3-D may be supported by the physiological effect of this agent as a norepinephrine releasing agent or norepinephrine reuptake

inhibitor. It is well described that norepinephrine has an effect on lipolysis,[42] which seems to occur via activation of hormone sensitive lipase, as well as through hormone sensitive lipase translocation from the cytosol to the lipid droplets within fat cells.[10] As just discussed, the reason that the addition of caffeine to the 1,3-D did not enhance this effect, but rather inhibited this effect, remains to be determined.

Despite some literature suggesting a potential antioxidant effect of caffeine[13,14] and 1,3-D,[15] we did not observe anything different in either MDA or NOx between conditions pre- or postexercise. However, it should be noted that the exercise itself did not result in an increase in these measures, thus making it difficult to detect any potential antioxidant benefit of either agent. As has been clearly presented in the literature, exercise has the potential to induce an oxidative stress, but this certainly does not occur in all circumstances.[16] In fact, many studies including exercise-trained subjects fail to note a significant increase in oxidative stress biomarkers. This was the case in the current investigation, despite the performance of a 10 km performance run. Our findings highlight the adaptive nature of the human body, with our exercise-trained subjects experiencing very little oxidative stress in a manner consistent with the principle of hormesis.[43]

Although no condition differences were noted for MDA or NOx, a condition effect was noted for TEAC, with the lowest values observed during the caffeine condition. However, it should be noted that the condition effect for TEAC appeared to be most influenced by pretreatment TEAC values (which were highest for the placebo condition and lowest for the caffeine condition), rather than by TEAC in response to treatment and exercise. In fact, the overall response curve for TEAC was very similar for all conditions (Fig. 2C). Although a decrease in TEAC may indicate an oxidative stress, considering the relatively minor change in this variable in response to exercise for all conditions, from a physiological point of view, we do not believe that the subtle condition differences noted for TEAC have significant meaning. This is underscored by the very similar TEAC values observed for all conditions at the 30 minute postexercise time, which were similar to or greater than pretreatment values for all conditions.

As expected, we noted an increase in all measured hemodynamic variables from pre- to postexercise. In addition, we noted a higher SBP with both the caffeine and 1,3-D conditions compared with placebo. Interestingly, the combination of caffeine + 1,3-D did not result in a higher SBP compared with placebo. In fact, the response was nearly identical to that of placebo—for both SBP and HR. At present, we admit that we have no explanation for these findings, with the possible exception of the following: Although 1,3-D is thought to promote vasoconstriction, caffeine may act to induce vasorelaxation.[18] It is possible that the combination of the two agents produced an acute vasorelaxation effect to allow for blood pressure to be reduced (in particular, considering that HR remained elevated above that for caffeine or 1,3-D alone). Future study is needed to further determine the independent and combined effects of these two agents on HR and blood pressure.

Although not identical in structure, it has been suggested that 1,3-D may have similar functional properties as ephedrine, which has been noted to exhibit mixed results in terms of HR and blood pressure, with the majority of work indicating a moderate increase in these variables.[44–47] Our previous work with 1,3-D and caffeine alone, while subjects remained at rest, have noted similar findings for SBP.[17] However, we noted an additive effect of the two agents when combined. This is in opposition to the findings of the current study, which obviously incorporated exercise into the design. Perhaps the alteration in vascular tone as a result of an acute exercise bout impacts the overall hemodynamic effects of subjects using a combination of caffeine + 1,3-D.

Finally, it should be reiterated that subjects were tested in a 10 hour fasted state, with the exception of the 30 g of maltodextrin provided within the conditions. It is possible that the hemodynamic effects could be attenuated if the conditions were ingested in a fed state, as may be typical for individuals using dietary supplements such as caffeine and 1,3-D. Moreover, the ingestion of a small standardized meal and/or the performance of testing later in the day may have allowed for better exercise performance, as such conditions may better mimic subjects' usual exercise routine. Of course, by incorporating this into the design, more variability would be introduced into the experiment.

Conclusions

We report for the first time that acute oral ingestion of caffeine and 1,3-D alone or in combination does not significantly improve exercise performance as measured by run time. Further, ingestion of either 1,3-D or caffeine alone results in the greatest increase in postexercise glycerol and FFA concentrations, whereas the combination of the two agents results in concentrations that are more similar to placebo. Neither agent has an impact on oxidative stress biomarkers, either before or after exercise. Finally, caffeine or 1,3-D alone increases SBP, without adversely impacting other hemodynamic variables. Future studies are needed using different dosages of these agents, as well as different exercise tests—possibly at a time of day more similar to subjects' usual training time, to determine whether more favorable effects can be observed after acute intake of caffeine or 1,3-D alone and in combination.

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Address correspondence to:
Richard J. Bloomer, Ph.D.
Cardiorespiratory/Metabolic Laboratory
Department of Health and Sport Sciences
106 Roane Field House
The University of Memphis
Memphis, TN 38152

E-mail: rbloomer@memphis.edu

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Hemodynamic and Hematologic Profile of Healthy Adults Ingesting Dietary Supplements Containing 1,3-Dimethylamylamine and Caffeine

Tyler M. Farney, Cameron G. McCarthy, Robert E. Canale, Rick J. Allman Jr. and Richard J. Bloomer

Cardiorespiratory/Metabolic Laboratory, Department of Health and Sport Sciences, University of Memphis, Memphis, TN, USA.
Corresponding author email: rbloomer@memphis.edu

Abstract

Background: 1,3-dimethylamylamine (a constituent of geranium), alone and in combination with caffeine, is widely used within dietary supplements. We have recently determined the hemodynamic effects of 1,3-dimethylamylamine and caffeine alone and in combination, using a single ingestion study. However, no study has determined the hemodynamic effects of these ingredients following chronic use. Moreover, no study has determined the effects of these ingredients on bloodborne variables related to health and safety. Therefore, the purpose of this investigation was to assess the hemodynamic and hematologic profile of two different dietary supplements containing 1,3-dimethylamylamine and caffeine (in addition to other ingredients), before and after two weeks of daily intake.

Methods: 7 men (24.9 ± 4.2 yrs) ingested the dietary supplement Jack3d™, while 4 men and 2 women (22.5 ± 1.8 yrs) ingested the dietary supplement OxyELITE Pro™ once per day for two weeks. On days 1 and 15, resting heart rate (HR), systolic (SBP), and diastolic (DBP) blood pressure were measured and rate pressure product (RPP) was calculated. Fasting blood samples were analyzed for complete blood counts, comprehensive metabolic panel, and lipid panel. These tests were done prior to ingestion of supplement. On days 1 and 15 following blood collection, subjects ingested the assigned supplement (2 servings) and HR, SBP, DBP, and RPP were recorded at 30, 60, 90, and 120 minutes post-ingestion.

Results: After 14 days of treatment, resting HR, SBP, DBP, and RPP were not increased ($P > 0.05$). No significant changes were noted in any measured bloodborne variable, with the exception of an increase in fasting blood glucose with ingestion of Jack3d™ ($P = 0.02$). In response to acute intake of the supplements, HR, DBP, and RPP were not increased statistically ($P > 0.05$). SBP was increased with OxyELITE Pro™ ($P = 0.03$), but not with Jack3d™ ($P = 0.09$). Compared to pre-ingestion and in general, both supplements resulted in an increase in SBP, DBP, and RPP from 5%–15%, with a peak occurring at the 60 or 90 minute post-ingestion time.

Conclusion: Acute ingestion of OxyELITE Pro™, but not Jack3d™, results in an increase in SBP. Chronic intake of two servings per day of OxyELITE Pro™ or Jack3d™ over a 14 day period does not result in an elevation in resting HR, SBP, DBP, or RPP. No significant changes are noted in any measured bloodborne variable following 14 days of ingestion, with the exception of blood glucose with Jack3d™. Longer term intervention studies inclusive of larger sample sizes are needed to extend these findings.

Keywords: 1,3-dimethylamylamine, caffeine, blood pressure, heart rate, blood

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Background

The use of nutritional supplements by the general population continues to rise, despite little direct scientific evidence in support of the use of many products.[1] Perhaps more importantly, few safety data are available pertaining to several of the ingredients commonly contained within finished products.[2] One such ingredient of interest is 1,3-dimethylamylamine, often combined with caffeine and other agents in an attempt to provide a stimulant "cocktail." The popularity of 1,3-dimethylamylamine continues to increase, as evidenced by its widespread use within many performance and lipolytic dietary supplements.

Suggested to be found within geranium,[3] 1,3-dimethylamylamine is widely used within the dietary supplement industry, in particular as a component of weight/fat loss products, as well as those targeting enhanced exercise performance. Unfortunately, very little is known about this ingredient, in particular as it pertains to oral ingestion by human subjects; something that we describe in detail in our recent report.[4]

In our prior work, we determined the effect of 1,3-dimethylamylamine alone (at two different dosages) and in combination with caffeine on the hemodynamic response to *acute intake* (2 hours post-ingestion).[4] In particular, we measured heart rate (HR), as well as systolic (SBP) and diastolic (DBP) blood pressure, and calculated rate pressure product (RPP) as an indicator of myocardial work. Our results indicated an increase in SBP in particular, with acute ingestion of 1,3-dimethylamylamine alone and in combination with caffeine. However, replication of this work is necessary. Moreover, to our knowledge, no study has determined the hemodynamic effects of 1,3-dimethylamylamine combined with caffeine following *chronic* use, which may differ from that observed with only single ingestion. Nor has any study determined the combined effects of these ingredients on bloodborne variables related to health and safety. Considering that many finished products now contain a combination of 1,3-dimethylamylamine and caffeine, and individuals using such products do so daily or semi-daily, we sought to determine the hemodynamic and hematologic profile of two different dietary supplements containing these ingredients before and after two weeks of daily intake. This was performed using an open label study design, with measurement of resting HR and blood pressure, in addition to bloodborne variables, on days 1 and 15. We also included a measurement of hemodynamic variables following acute intake of the supplements on days 1 and 15. In terms of acute intake, we hypothesized that the supplements would increase blood pressure, in particular SBP, as noted in our previous work. In terms of chronic intake, we hypothesized that no significant changes would be noted in any measured variable, due to the acute nature of the stimulant effect.

Methods

Subjects

Healthy men and women participated in this study. All subjects completed a medical history and physical activity questionnaire to determine eligibility. No subject smoked cigarettes or had self reported diagnosed disease of cardiovascular or metabolic origin. Subjects were active and considered to be exercise-trained, as they performed aerobic (eg, jogging, running, cycling, swimming) and anaerobic (eg, sprinting, resistance training) exercise for the past several years (based on self report). Subject descriptive characteristics are presented in Table 1. All experimental procedures were performed in accordance with the Helsinki Declaration. The University of Memphis Human Subjects Committee approved all experimental procedures (H10-77 and H10-78). Subjects provided verbal and written consent prior to participating in this study.

Conditions and testing

Following all screening procedures, subjects reported to the lab in the morning hours (0600–0900) on two different occasions separated by 14 days. The time of day for testing was matched for each subject. Procedures described below were identical for both test sessions (and for both supplements tested). The dietary supplements used in this investigation were provided by USPlabs (Dallas, TX) and contained a combination of 1,3-dimethylamylamine and caffeine, in addition to other ingredients which may have contributed to the measured effect. Specifically the two supplements used were: (1) OxyELITE Pro™ (containing a proprietary blend of bauhinia purpurea, bacopa monniera, 1,3-dimethylamylamine, cirsium oligophyllum, rauwolscine extract, and caffeine [100 mg per capsule]) and (2) Jack3d™ (containing a proprietary blend of arginine alpha-ketoglutarate, creatine monohydrate, beta alanine,

Table 1. Characteristics of subjects using OxyELITE Pro™ and Jack3d™.

Variable	OxyELITE Pro™ (N = 6: 4 men and 2 women)	Jack3d™ (N = 7 men)
Age (yrs)	22.5 ± 1.8	24.9 ± 4.2
Height (cm)	173.8 ± 12.5	178.2 ± 8.6
Weight (kg)	65.9 ± 8.6	83.8 ± 9.4
BMI ($kg \cdot m^{-2}$)	21.9 ± 2.1	26.5 ± 3.3
Body fat (%)	13.8 ± 9.7	14.3 ± 5.2
Waist (cm)	72.8 ± 3.8	86.4 ± 7.9
Hip (cm)	95.4 ± 5.2	103.7 ± 5.5
Waist:hip	0.76 ± 0.04	0.83 ± 0.05
Resting heart rate (bpm)*	67.6 ± 9.7	60.1 ± 5.2
Resting systolic blood pressure (mmHg)*	104.0 ± 5.5	112.6 ± 13.8
Resting diastolic blood pressure (mmHg)*	63.8 ± 9.7	64.3 ± 8.0
Years anaerobic exercise training	1.6 ± 1.9	7.1 ± 6.5
Hours per week anaerobic exercise	1.8 ± 2.1	6.8 ± 2.4
Years aerobic exercise training	5.8 ± 5.6	4.1 ± 5.2
Hours per week aerobic exercise	3.5 ± 1.8	1.5 ± 1.3

Notes: Data are mean ± SD. *Measured during screening visit; prior to pre (day 1) testing.

caffeine, 1,3-dimethylamylamine, and schizandrol A). Supplements were produced in accordance with Good Manufacturing Practices. For OxyELITE Pro™, subjects ingested two servings/capsules of the dietary supplement (both during the acute intake assessment [on days 1 and 15] and each day during the chronic intake period). For Jack3d™, subjects ingested two servings/scoops of the dietary supplement mixed in water (both during the acute intake assessment [on days 1 and 15] and each day during the chronic intake period). This was an open label study. No placebo conditions were included in this initial investigation, as we were mainly interested in potential changes in our measured variables across time (both with the acute intake study and with the chronic intake study). This lack of a placebo condition is indeed a limitation of the present design. Subjects' self-report of daily intake, coupled with our collection of remaining supplements at the conclusion of the 14-day supplementation period, determined compliance to supplementation.

On days 1 and 15 (pre and post intervention), subjects reported to the laboratory in a fasted state (≥10 hours), without caffeine consumption during the past 10 hours. Subjects were asked not to exercise or to perform any strenuous physical activity for the 24 hours prior to each testing day. Following a 10 minute rest period, HR (via 60 second palpation of the radial artery), as well as SBP and DBP (via auscultation using a dual-earpiece stethoscope by two trained technicians) were measured. RPP was calculated as: HR × SBP. A blood sample was then obtained. These tests (HR, blood pressure, and blood) were done prior to ingestion of the supplements. Subjects then ingested the assigned supplement in the presence of an investigator. At 30, 60, 90, and 120 minutes post-ingestion, HR, SBP, and DBP were measured, and RPP was calculated. Subjects remained in the lab and rested during this time (read, watched television, worked on the computer, listened to music) and no food was allowed during the two hour post intake period. However, water was allowed *ad libitum*, and was measured and matched for each subject on both days of testing. Following testing on day 1, subjects were provided with their assigned supplement and given instructions on how/when to ingest (ie, 2 capsules or 2 scoops daily—preferably ≥6 hours prior to bedtime).

Blood collection and biochemistry

A total of two venous blood samples were taken from subjects during the course of the study: day 1 and day 15. Blood was processed and sent to Laboratory Corporation of America for analysis of complete blood count, comprehensive metabolic panel, and lipid panel. These measures were included for a comprehensive assessment of bloodborne variables, as is routinely performed as part of a physical examination.

They were also included within the design, as this is common practice within many dietary supplement studies focused on blood related "safety" variables. We had no directional hypotheses related to the impact of the supplements on these measures. The complete blood count was determined using an automated cell counter (Coulter LH750). The comprehensive metabolic panel was determined using automated procedures (Roche/Hitachi Modular). The lipid panel was determined using enzymatic procedures (Roche/Hitachi Modular).

Dietary intake and physical activity

Subjects were asked to maintain their usual diet and physical activity patterns over the course of the entire two week study period. However, they were asked to refrain from strenuous activity during the 24 hours prior to each test day. As the tested dietary supplements have been noted anecdotally to result in appetite suppression, subjects were asked to rate their overall appetite before and after the intervention using a visual analog scale (0 = no hunger at all; 10 = extreme hunger).

Statistical analysis

Resting, pre/post intervention data (hemodynamic, bloodborne, and appetite) for both supplements were compared using a one way analysis of variance (ANOVA); a statistical technique used to evaluate whether there are differences between the mean values across different groups. In regards to the acute intake study, a 2 (pre/post intervention) × 5 (time) ANOVA was used. For the pre/post intervention effect to be significant ($P \leq 0.05$), a measureable difference was observed between day 1 and day 15 data. Likewise, for the time effect to be significant ($P \leq 0.05$), a measureable difference was observed between data collected at the various times before and following acute intake of the supplements (Pre, 30, 60, 90, and 120 minutes post-ingestion). Percent change values for all hemodynamic data were also calculated and compared using a 2 (pre/post intervention) × 5 (time) ANOVA. Tukey post hoc testing was used when needed, to determine *where* specific differences may have existed. No attempt was made to compare data between the two supplements, as we were attempting to characterize the effects of using supplements containing 1,3-dimethylamylamine and caffeine, and not make direct comparisons between such supplements. All analyses were performed using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC). Statistical significance was set at $P \leq 0.05$. The data are presented as mean ± SEM, except for subject descriptive characteristics (mean ± SD).

Results

Appetite data

Appetite was lower from pre (6.3 ± 0.5) to post (4.3 ± 0.6) intervention for OxyELITE Pro™ ($P = 0.04$). However, appetite was not different from pre (6.1 ± 0.1) to post (6.1 ± 0.5) intervention for Jack3d™ ($P = 1.00$). It is possible that ingredients aside from 1,3-dimethylamylamine and caffeine, which are present within OxyELITE Pro™ but not within Jack3d™, may have contributed to these findings.

Resting data (pre-supplementation): hemodynamic and biochemical

When determined prior to the acute intake of the supplements, no change from pre (day 1) to post (day 15) intervention was noted for HR (Table 2), SBP (Table 3), DBP (Table 4), or RPP (Table 5) for subjects ingesting either supplement for the two week period ($P > 0.05$). In fact, a slight (non-statistical) reduction in HR, SBP, and RPP were observed following treatment with OxyELITE Pro™. No significant changes ($P > 0.05$) were observed in complete blood count (Table 6) or lipid panel (Table 7) data for either supplement. Likewise, no significant changes ($P > 0.05$) were noted in comprehensive metabolic panel data for either supplement (Table 8), with the exception of blood glucose with ingestion of Jack3d™ ($P = 0.02$).

Acute intake of supplement: hemodynamics

Following acute intake of the supplements, with regards to HR, the following effects were observed: For OxyELITE Pro™, a pre/post intervention effect was noted ($P = 0.008$), with values lower post intervention compared to pre intervention. No time ($P = 0.46$) or pre/post intervention × time effect was noted ($P = 0.95$). For Jack3d™, no pre/post intervention ($P = 0.83$), time ($P = 0.22$), or pre/post intervention × time effect was noted ($P = 0.88$). When expressing

Table 2. Heart rate (bpm) before and following ingestion of OxyELITE Pro™ or Jack3d™ pre (day 1) and post (day 15) daily supplement use.

Time	OxyELITE Pro™		Jack3d™	
	Pre (day 1)*	Post (day 15)	Pre (day 1)	Post (day 15)
Pre	64.8 ± 2.9	60.9 ± 2.6	58.3 ± 2.7	60.4 ± 2.5
30 min	62.8 ± 2.7	56.1 ± 2.8	55.6 ± 2.2	58.4 ± 3.2
60 min	68.8 ± 6.1	61.7 ± 2.4	61.4 ± 2.1	59.1 ± 2.2
90 min	71.4 ± 6.9	60.9 ± 3.6	59.7 ± 2.0	59.0 ± 2.4
120 min	68.8 ± 4.8	61.9 ± 3.1	62.1 ± 0.9	60.6 ± 2.3

Notes: Data are mean ± SEM. OxyELITE Pro™: *Pre/Post intervention ($P = 0.008$; Pre intervention higher than Post intervention), Time ($P = 0.46$); Pre/Post intervention x Time ($P = 0.95$). Jack3d™: Pre/Post intervention ($P = 0.83$), Time ($P = 0.22$); Pre/Post intervention x Time ($P = 0.88$).

Table 3. Systolic blood pressure (mmHg) before and following ingestion of OxyELITE Pro™ or Jack3d™ pre (day 1) and post (day 15) daily supplement use.

Time	OxyELITE Pro™		Jack3d™	
	Pre (day 1)	Post (day 15)	Pre (day 1)	Post (day 15)
Pre	103.0 ± 4.0	99.0 ± 5.4	109.0 ± 2.9	109.3 ± 3.3
30 min	106.9 ± 7.5	104.1 ± 4.2	112.4 ± 6.3	122.3 ± 3.1
60 min*	118.1 ± 5.1	113.0 ± 4.1	121.7 ± 5.1	121.4 ± 5.4
90 min*	119.2 ± 3.6	110.2 ± 5.8	115.7 ± 4.8	122.7 ± 3.1
120 min*	115.5 ± 5.2	110.0 ± 5.5	115.7 ± 5.4	122.1 ± 5.7

Notes: Data are mean ± SEM. OxyELITE Pro™: Pre/Post intervention ($P = 0.11$), *Time ($P = 0.03$; 60, 90, and 120 min Post-ingestion higher than Pre [$P < 0.05$]); Pre/Post intervention x Time ($P = 0.98$). Jack3d™: Pre/Post intervention ($P = 0.12$), Time ($P = 0.09$); Pre/Post intervention x Time ($P = 0.77$).

Table 4. Diastolic blood pressure (mmHg) before and following ingestion of OxyELITE Pro™ or Jack3d™ pre (day 1) and post (day 15) daily supplement use.

Time	OxyELITE Pro™		Jack3d™	
	Pre (day 1)	Post (day 15)	Pre (day 1)	Post (day 15)
Pre	63.0 ± 3.8	62.0 ± 1.5	63.3 ± 3.3	64.6 ± 4.1
30 min	66.3 ± 4.9	63.8 ± 2.0	68.9 ± 5.3	74.0 ± 2.2
60 min	69.2 ± 5.1	68.3 ± 2.2	71.7 ± 5.0	73.7 ± 4.8
90 min	67.1 ± 6.1	65.7 ± 2.7	68.4 ± 3.5	75.1 ± 1.8
120 min	66.1 ± 4.2	65.2 ± 3.7	70.4 ± 5.2	72.7 ± 3.6

Notes: Data are mean ± SEM. OxyELITE Pro™: Pre/Post intervention ($P = 0.59$), Time ($P = 0.62$); Pre/Post intervention x Time ($P = 0.99$). Jack3d™: Pre/Post intervention ($P = 0.18$), Time ($P = 0.19$); Pre/Post intervention x Time ($P = 0.96$).

Table 5. Rate pressure product before and following ingestion of OxyELITE Pro™ or Jack3d™ pre (day 1) and post (day 15) daily supplement use.

Time	OxyELITE Pro™		Jack3d™	
	Pre (day 1)*	Post (day 15)	Pre (day 1)	Post (day 15)
Pre	6634.3 ± 193.4	6033.7 ± 442.1	6381.3 ± 398.9	6606.6 ± 343.3
30 min	6778.3 ± 733.9	5840.3 ± 393.8	6309.3 ± 545.9	6920.6 ± 481.0
60 min	8228.7 ± 1068.8	6938.8 ± 245.0	7496.9 ± 463.0	7209.7 ± 470.2
90 min	8573.1 ± 977.9	6637.8 ± 330.9	6935.1 ± 432.5	7268.6 ± 413.4
120 min	8053.4 ± 907.4	6766.8 ± 363.5	7207.6 ± 411.2	7454.9 ± 486.9

Notes: Data are mean ± SEM. OxyELITE Pro™: *Pre/Post intervention ($P = 0.004$; Pre intervention higher than Post intervention), Time ($P = 0.09$); Pre/Post intervention x Time ($P = 0.88$). Jack3d™: Pre/Post intervention ($P = 0.44$), Time ($P = 0.20$); Pre/Post intervention x Time ($P = 0.91$).

Table 6. Complete blood count data pre (day 1) and post (day 15) daily intake of OxyELITE Pro™ or Jack3d™.

Variable	OxyELITE Pro™		Jack3d™	
	Pre (day 1)	Post (day 15)	Pre (day 1)	Post (day 15)
WBC ($10^3 \cdot \mu L^{-1}$)	6.0 ± 0.4	6.1 ± 0.4	5.3 ± 0.3	5.1 ± 0.3
RBC ($10^6 \cdot \mu L^{-1}$)	4.3 ± 0.1	4.2 ± 0.2	4.6 ± 0.1	4.6 ± 0.1
Hemoglobin ($g \cdot dL^{-1}$)	13.4 ± 0.4	13.2 ± 0.4	14.6 ± 0.3	14.5 ± 0.2
Hematocrit (%)	39.2 ± 1.1	38.9 ± 1.6	41.7 ± 0.8	42.3 ± 0.7
MCV (fL)	92.0 ± 1.1	93.0 ± 1.7	90.0 ± 1.7	92.3 ± 1.3
MCH (pg)	31.4 ± 0.5	31.4 ± 0.6	31.5 ± 0.5	31.7 ± 0.5
MCHC ($g \cdot dL^{-1}$)	34.2 ± 0.4	33.9 ± 0.4	35.0 ± 0.2	34.3 ± 0.3
RDW (%)	13.3 ± 0.3	13.3 ± 0.3	12.9 ± 0.2	13.5 ± 0.3
Platelets ($10^3\ \mu L^{-1}$)	196.2 ± 14.8	198.3 ± 19.3	193.6 ± 12.9	198.4 ± 14.2
Neutrophils (%)	50.0 ± 2.4	51.5 ± 3.8	50.6 ± 2.6	47.0 ± 3.2
Lymphocytes (%)	40.3 ± 2.2	38.7 ± 4.0	36.4 ± 2.4	38.7 ± 3.1
Monocytes (%)	7.2 ± 0.6	6.7 ± 0.7	8.3 ± 0.5	8.9 ± 0.7
Eosinophils (%)	2.0 ± 0.4	2.5 ± 0.5	4.1 ± 0.7	4.9 ± 0.7
Basophils (%)	0.5 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2

Notes: Values are mean ± SEM. OxyELITE Pro™: No significant difference noted from pre to post for any variable ($P > 0.05$). Jack3d™: No significant difference noted from pre to post for any variable ($P > 0.05$).

data as percent change from pre-ingestion, the following effects were noted: For OxyELITE Pro™, no pre/post intervention ($P = 0.17$), time ($P = 0.41$), or pre/post intervention × time effect was noted ($P = 0.94$). For Jack3d™, a pre/post intervention effect was noted ($P = 0.01$), with a lower response post intervention compared to pre intervention. A time effect was also noted ($P = 0.01$), with values at 120 minutes different than values at 30 minutes ($P < 0.05$). No pre/post intervention × time effect was noted ($P = 0.59$). Heart rate data are presented in Table 2 and Figure 1.

With regards to SBP, the following effects were observed: For OxyELITE Pro™, no pre/post intervention ($P = 0.11$) or pre/post intervention × time effect was noted ($P = 0.98$). However, a time effect was noted ($P = 0.03$), with values higher than pre-ingestion at 60, 90, and 120 minutes post-ingestion ($P < 0.05$). For Jack3d™, no pre/post intervention ($P = 0.12$), time ($P = 0.09$), or pre/post intervention × time effect was noted ($P = 0.77$). When expressing data as percent change from pre-ingestion, the following effects were noted: For OxyELITE Pro™, no pre/post intervention ($P = 0.84$) or pre/post intervention × time effect was noted ($P = 0.92$). However, a time effect was noted ($P = 0.0004$), with values higher than pre-ingestion at 60, 90, and 120 minutes post-ingestion ($P < 0.05$). For Jack3d™, a pre/post intervention effect was noted ($P = 0.04$), with a greater response post intervention compared to pre intervention. A time effect was also noted ($P = 0.006$), with values higher than pre-ingestion at 60, 90, and 120 minutes post-ingestion

Table 7. Lipid panel data pre (day 1) and post (day 15) daily intake of OxyELITE Pro™ or Jack3d™.

Variable	OxyELITE Pro™		Jack3d™	
	Pre (day 1)	Post (day 15)	Pre (day 1)	Post (day 15)
Cholesterol ($mg \cdot dL^{-1}$)	164.5 ± 13.2	170.0 ± 14.5	145.1 ± 8.1	147.6 ± 8.8
Triglycerides ($mg \cdot dL^{-1}$)	87.8 ± 16.3	70.3 ± 9.1	74.1 ± 11.3	80.0 ± 11.3
HDL-C ($mg \cdot dL^{-1}$)	66.8 ± 5.9	69.3 ± 6.9	50.7 ± 4.7	52.3 ± 3.5
VLDL-C ($mg \cdot dL^{-1}$)	17.5 ± 3.2	14.2 ± 1.8	14.7 ± 2.2	16.0 ± 2.2
LDL-C ($mg \cdot dL^{-1}$)	80.2 ± 6.7	86.5 ± 9.0	79.7 ± 6.2	79.3 ± 7.7
LDL-C/HDL-C	1.2 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.6 ± 0.2
Total:HDL-C	2.5 ± 0.1	2.5 ± 0.2	2.9 ± 0.2	2.9 ± 0.2

Notes: Values are mean ± SEM. OxyELITE Pro™: No significant difference noted from pre to post for any variable ($P > 0.05$). Jack3d™: No significant difference noted from pre to post for any variable ($P > 0.05$).

Table 8. Metabolic panel data pre (day 1) and post (day 15) daily intake of OxyELITE Pro™ or Jack3d™.

Variable	OxyELITE Pro™		Jack3d™	
	Pre (day 1)	Post (day 15)	Pre (day 1)	Post (day 15)
Glucose ($mg \cdot dL^{-1}$)	78.2 ± 4.4	77.0 ± 3.1	86.4 ± 2.3*	94.6 ± 2.0
BUN ($mg \cdot dL^{-1}$)	18.8 ± 3.9	17.0 ± 2.5	14.9 ± 1.1	16.9 ± 0.9
Creatinine ($mg \cdot dL^{-1}$)	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.0	1.2 ± 0.0
BUN:creatinine	16.3 ± 2.8	17.2 ± 2.5	13.1 ± 1.8	13.9 ± 0.7
Sodium ($mmol \cdot L^{-1}$)	138.2 ± 0.8	141.0 ± 1.6	141.1 ± 0.4	140.7 ± 0.4
Potassium ($mmol \cdot L^{-1}$)	4.7 ± 0.2	4.8 ± 0.4	4.7 ± 0.2	4.8 ± 0.2
Chloride ($mmol \cdot L^{-1}$)	101.3 ± 0.6	102.5 ± 1.1	102.1 ± 0.5	103.3 ± 0.5
CO_2 ($mmol \cdot L^{-1}$)	28.5 ± 0.9	27.2 ± 0.9	28.1 ± 0.5	28.6 ± 0.6
Calcium ($mg \cdot dL^{-1}$)	9.1 ± 0.1	9.3 ± 0.2	9.3 ± 0.1	9.4 ± 0.1
Protein ($g \cdot dL^{-1}$)	6.7 ± 0.2	6.6 ± 0.2	6.6 ± 0.1	6.6 ± 0.1
Albumin ($g \cdot dL^{-1}$)	4.4 ± 0.2	4.4 ± 0.2	4.5 ± 0.0	4.3 ± 0.1
Globulin ($g \cdot dL^{-1}$)	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1
A:G	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1
Bilirubin ($mg \cdot dL^{-1}$)	0.4 ± 0.1	0.5 ± 0.1	1.0 ± 0.1	0.8 ± 0.2
Alk Phos ($IU \cdot L^{-1}$)	64.2 ± 3.9	60.3 ± 4.4	64.1 ± 7.9	63.6 ± 8.4
AST (SGOT) ($IU \cdot L^{-1}$)	21.0 ± 2.2	25.7 ± 5.3	25.1 ± 2.6	30.7 ± 2.5
ALT (SGPT) ($IU \cdot L^{-1}$)	19.0 ± 1.9	16.8 ± 1.9	23.7 ± 2.8	27.6 ± 3.3
GGT ($IU \cdot L^{-1}$)	22.1 ± 2.9	20.6 ± 3.2	16.1 ± 1.2	19.9 ± 2.3

Notes: Values are mean ± SEM. OxyELITE Pro™: No significant difference noted from pre to post for any variable ($P > 0.05$). Jack3d™: *Significant difference noted from pre to post for glucose ($P = 0.02$). No other significant difference noted from pre to post for any variable ($P > 0.05$).

($P < 0.05$). No pre/post intervention × time effect was noted ($P = 0.44$). Systolic blood pressure data are presented in Table 3 and Figure 2.

With regards to DBP, the following effects were observed: For OxyELITE Pro™, no pre/post intervention ($P = 0.59$), time (0.62), or pre/post intervention × time effect was noted ($P = 0.90$). For Jack3d™, no pre/post intervention ($P = 0.18$), time ($P = 0.19$), or pre/post intervention × time effect was noted ($P = 0.96$). When expressing data as percent change from pre-ingestion,

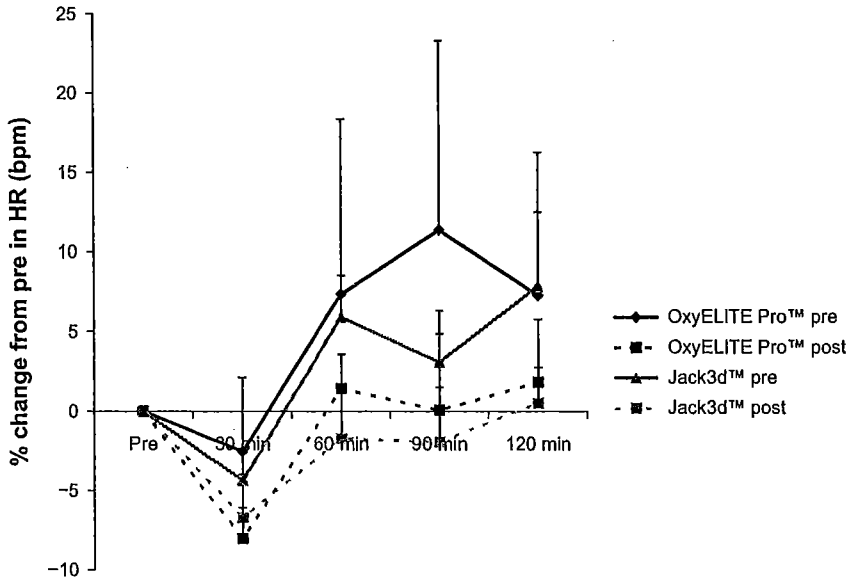

Figure 1. Percent change from pre in heart rate for OxyELITE Pro™ and Jack3d™ pre (day 1) and post (day 15) daily supplement use.
Notes: Data are mean ± SEM. OxyELITE Pro™: Pre/Post intervention ($P = 0.17$), Time ($P = 0.41$); Pre/Post intervention × Time ($P = 0.94$). Jack3d™: Pre/Post intervention ($P = 0.01$), Time ($P = 0.01$); Pre/Post intervention × Time ($P = 0.59$).

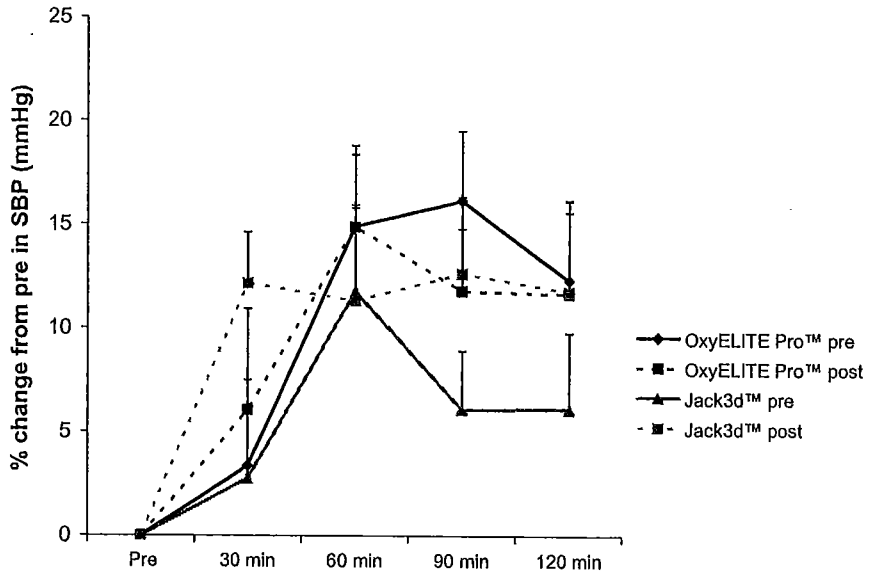

Figure 2. Percent change from pre in systolic blood pressure for OxyELITE Pro™ and Jack3d™ pre (day 1) and post (day 15) daily supplement use.
Notes: Data are mean ± SEM. OxyELITE Pro™: Pre/Post intervention ($P = 0.84$), Time ($P = 0.0004$); Pre/Post intervention × Time ($P = 0.92$). Jack3d™: Pre/Post intervention ($P = 0.04$), Time ($P = 0.006$); Pre/Post intervention × Time ($P = 0.44$).

the following effects were noted: For OxyELITE Pro™, no pre/post intervention ($P = 0.71$), time ($P = 0.40$), or pre/post intervention × time effect was noted ($P = 0.99$). For Jack3d™, no pre/post intervention ($P = 0.34$), time ($P = 0.20$), or pre/post intervention × time effect was noted ($P = 0.95$). Diastolic blood pressure data are presented in Table 4 and Figure 3.

With regards to RPP, the following effects were observed: For OxyELITE Pro™, a pre/post intervention effect was noted ($P = 0.004$), with values

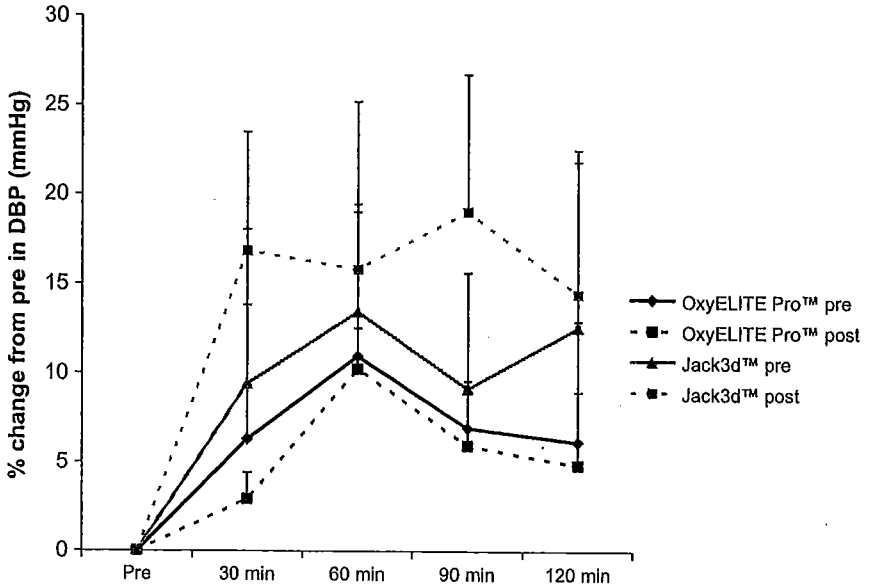

Figure 3. Percent change from pre in diastolic blood pressure for OxyELITE Pro™ and Jack3d™ pre (day 1) and post (day 15) daily supplement use.
Notes: Data are mean ± SEM. OxyELITE Pro™: Pre/Post intervention ($P = 0.71$), Time ($P = 0.40$); Pre/Post intervention × Time ($P = 0.99$). Jack3d™: Pre/Post intervention ($P = 0.34$). Time ($P = 0.20$), Pre/Post intervention × Time ($P = 0.95$).

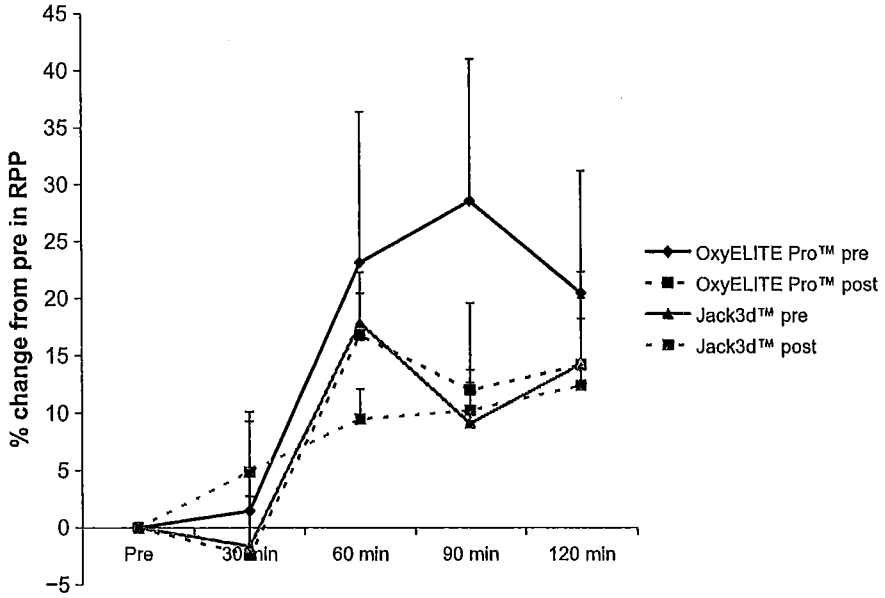

Figure 4. Percent change from pre in rate pressure product for OxyELITE Pro™ and Jack3d™ pre (day 1) and post (day 15) daily supplement use.
Notes: Data are mean ± SEM. OxyELITE Pro™: Pre/Post intervention ($P = 0.22$), Time ($P = 0.02$); Pre/Post intervention × Time ($P = 0.99$). Jack3d™: Pre/Post intervention ($P = 0.85$), Time ($P = 0.001$); Pre/Post intervention × Time ($P = 0.48$).

lower post intervention compared to pre intervention. No time ($P = 0.09$) or pre/post intervention × time effect was noted ($P = 0.88$). For Jack3d™, no pre/post intervention ($P = 0.44$), time ($P = 0.20$), or pre/post intervention × time effect was noted ($P = 0.91$). When expressing data as percent change from pre-ingestion, the following effects were noted: For OxyELITE Pro™, no pre/post intervention ($P = 0.22$) or pre/post intervention × time effect was noted ($P = 0.99$). However, a time effect was noted ($P = 0.02$), with values higher than pre-ingestion at 60, 90, and 120 minutes post-ingestion ($P < 0.05$). For Jack3d™, no pre/post intervention ($P = 0.85$) or pre/post intervention × time effect was noted ($P = 0.48$). However, a time effect was noted ($P = 0.001$), with values higher than pre-ingestion at 60 and 120 minutes post-ingestion ($P < 0.05$). Rate pressure product data are presented in Table 5 and Figure 4.

Discussion

This study is the first to report the hemodynamic and hematologic effects of acute and chronic intake of dietary supplements containing a combination of 1,3-dimethylamylamine and caffeine in human subjects. Our data indicate that acute ingestion of the tested dietary supplements results in an increase in myocardial work (as measured specifically by SBP), which agree with our prior work using the isolated ingredients 1,3-dimethylamylamine and caffeine alone and in combination.[4] It should be noted that the present findings are specific to an ingested dosage of two servings of the supplements. As we have noted in our prior work with 1,3-dimethylamylamine and caffeine, the increase in SBP is dose dependent.[4] Therefore, use of only one serving of the tested supplements would likely result in less of a response as compared to what we report here. This is underscored by prior work indicating a dose-dependent effect for caffeine.[6,7] Considering that 1,3-dimethylamylamine and caffeine are chief ingredients within both of the tested supplements, lowering the dosage would likely result in a lowering of the blood pressure response to acute ingestion. However, regardless of dose, if an increase in SBP was observed following chronic exposure to such dietary supplements, this may have clinical relevance in terms of increasing the risk for cardiovascular disease.[5] We address this with our results for the 14-day intervention period, which indicate that no increase in resting HR or blood pressure is observed, nor are bloodborne variables negatively impacted with either supplement (with the possible exception of blood glucose with Jack3d™,

as discussed below). These findings are underscored by our recent placebo controlled studies involving a 10-week intervention with Jack3d™ (with subjects using the product only on exercise training days) and an 8-week intervention involving daily intake of OxyELITEPro™, indicating similar findings as presented within for the 2-week intervention. That is, similar effects were noted across the intervention period for resting blood pressure and bloodborne safety variables for supplements and placebo (unpublished findings).

While no changes were noted in any bloodborne variable within the complete blood count and lipid panel, an increase was noted in fasting blood glucose from pre to post intervention with Jack3d™ treatment (Table 8). While caffeine intake is known to alter glucose homeostasis,[8] this finding cannot be solely explained based on the caffeine (or the 1,3-dimethylamylamine), as similar findings would also be expected for subjects using OxyELITE Pro™ (unless other ingredients within OxyELITE Pro™ counteracted this effect). Therefore, it is possible that the creatine monohydrate within the Jack3d™ may be responsible for this effect. In support of this hypothesis, at least one published report documents the influence of creatine on glucose homeostasis in human subjects,[9] by noting that supplemental creatine monohydrate at a daily dosage of 5 grams/day for 42 days resulted in a trend ($P = 0.07$) towards elevated fasting plasma glucose levels. If this were the case, this may be expected for all products containing creatine monohydrate. Aside from creatine, we are unaware of any data linking ingestion of the other ingredients contained within Jack3d™ (arginine alpha-ketoglutarate, beta alanine, schizandrol A) with impairments in glucose homeostasis. Moreover, our recent 10-week intervention study involving Jack3d™ did not result in an increase in fasting blood glucose, suggesting that the results obtained in the present study may have been influenced by confounding factors.

As noted in the Methods section, both of the supplements tested in this study contain additional ingredients that may be at least partly responsible for the noted findings. However, when comparing the findings for hemodynamic measures with those from our prior study using 1,3-dimethylamylamine alone and in combination with caffeine,[4] the results are relatively similar. Coupled with the fact that many of the other ingredients included within these products are not known stimulants, it is likely that the simple combination of 1,3-dimethylamylamine and caffeine are responsible for the majority of the effect. It should also be noted that 1,3-dimethylamylamine is clearly responsible for some of the effect on the measured parameters, as caffeine alone does not result in the same magnitude of increase in SBP and RPP as compared to what we observed here.[4,10]

Although the effect of caffeine on cardiac rate and systemic blood pressure is well described,[10] the same is not true for 1,3-dimethylamylamine, with only one published report documenting these effects.[4] A suggested component of geranium,[3] 1,3-dimethylamylamine is becoming increasingly popular as a component of dietary supplements, in particular those targeting weight/fat loss and enhanced exercise performance. The action of 1,3-dimethylamylamine is cited as a simple aliphatic amine, functioning as a norepinephrine reuptake inhibitor and/or norepinephrine releasing agent. It is also suggested to stimulate smooth muscle and act as a vasoconstrictor.[4] These effects certainly help to explain the elevation in blood pressure in response to acute ingestion. Anecdotal reports indicate a potential effect on appetite suppression, which is at least partially supported by our findings of decreased appetite with OxyELITE Pro™ treatment. However, no effect was noted for appetite in subjects using Jack3d™. Clearly, additional studies are needed to confirm our findings and to provide mechanistic data pertaining to the effect of 1,3-dimethylamylamine on hemodynamics, appetite, and associated parameters.

As expected based on the pharmacologic profiles of caffeine and of 1,3-dimethylamylamine, *acute* intake of dietary supplements containing these agents results in an increase in myocardial work. Specifically, SBP is increased significantly in response to treatment, while DBP, and RPP increase to a lesser extent. While such changes are common with weight loss supplements,[11–16] in particular for those including caffeine and other stimulants, individuals using dietary supplements containing 1,3-dimethylamylamine alone or in combination with other stimulants should be advised to carefully follow label recommendations pertaining to dosing, and only use such products under the guidance of a qualified healthcare provider.

Based on our data, which admittedly involved a very small number of subjects, it appears that such products should be avoided by individuals who are hypertensive (resting blood pressure ≥140/90 mmHg) or those who are pre-hypertensive (resting blood pressure ≥120/80 mmHg).

Finally, it is worth noting the various comments provided by subjects involved in this study. Related to the use of OxyELITE Pro™, comments included: heightened sense of focus and energy, improved workout intensity, sleeplessness, shakiness, feeling anxious, feeling of chills, sweating, nausea, tingling, and feeling of fatigue. Four subjects (3 women and 1 man) who began treatment with OxyELITE Pro™ decided not to complete the intervention period due to dislike for the supplement (sleeplessness, inability to focus, nausea, headaches, jittery). It should be noted that 2 of these 4 individuals had newly acquired employment obligations for which they were not willing to interfere with. Hence, by their own admission, any change in their usual daily routine which they may have attributed to intake of the OxyELITE Pro™ was deemed unacceptable. Four additional subjects were recruited in order to replace these subjects. Data are available only for those subjects who completed the study. Related to the use of Jack3d™, comments included: talkative, heightened sense of focus and energy, improved workout intensity, sleeplessness, feeling anxious, feeling of chills, tingling, and feeling of fatigue. Subjects tolerated Jack3d™ well and all who began treatment completed the entire two week intervention. While no severe adverse events were noted with either supplement, the above comments should be considered along with the objective data presented, as some individuals may find such effects unacceptable.

Conclusion

In conclusion, we report that the finished products OxyELITE Pro™ and Jack3d™, both of which contain a combination of 1,3-dimethylamylamine and caffeine, do not elevate resting HR, SBP, DBP, or RPP when ingested daily for 14 days. Moreover, no significant changes were noted in any measured bloodborne variable following 14 days of ingestion, with the exception of blood glucose with ingestion of Jack3d™. Acute ingestion of these products results in an increase in SBP, while leading to a statistically insignificant increase in DBP and RPP. Considering that these data were generated using two servings of the supplements, it is likely that a single serving would have resulted in a lesser change. Additional acute studies are needed to replicate our findings. In addition, intervention studies involving a larger subject sample monitored over an extended period of time are needed to more fully elucidate the effects of 1,3-dimethylamylamine and caffeine on hemodynamic and hematologic variables.

Competing Interests

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

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

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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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Nutrition and Metabolic Insights

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ORIGINAL RESEARCH

A Finished Dietary Supplement Stimulates Lipolysis and Metabolic Rate in Young Men and Women

Cameron G. McCarthy, Tyler M. Farney, Robert E. Canale, Rick J. Alleman Jr. and Richard J. Bloomer

Cardiorespiratory/Metabolic Laboratory, Department of Health and Sport Sciences, University of Memphis, Memphis, TN, USA. Corresponding author email: rbloomer@memphis.edu

Abstract

Background: Dietary supplements are often marketed to increase lipolysis and thermogenesis, with the proposed end result being weight loss and body fat reduction. It was the purpose of the present investigation to study the acute effects of a weight/fat loss supplement within a sample of healthy human subjects.

Methods: Twelve subjects (men 24.8 ± 4.3 yrs; women 22.8 ± 0.4 yrs) ingested a dietary supplement (OxyELITE Pro™) or a placebo, on two separate days in a double-blind, cross-over design. Blood samples were collected immediately before ingestion, and at 60 and 120 minutes post ingestion, and analyzed for plasma glycerol and free fatty acids (FFA). Breath samples were collected immediately before ingestion and at 30, 60, 90, and 120 minutes post ingestion, for a measure of kilocalorie expenditure using indirect calorimetry. Area under the curve (AUC) was calculated. Heart rate and blood pressure were recorded at all times and rate pressure product (RPP) was calculated.

Results: AUC was greater for supplement compared to placebo for glycerol ($22.74 \pm 1.98\ \mu g \cdot mL^{-1} \cdot 2\ hr^{-1}$ vs. $15.76 \pm 1.36\ \mu g \cdot mL^{-1} \cdot 2\ hr^{-1}$; $P = 0.001$), FFA ($1.62 \pm 0.07\ mmol \cdot L^{-1} \cdot 2\ hr^{-1}$ vs. $0.78 \pm 0.12\ mmol \cdot L^{-1} \cdot 2\ hr^{-1}$; $P < 0.0001$), and kilocalorie expenditure ($149 \pm 7\ kcal \cdot 2\ hr^{-1}$ vs. $122 \pm 8\ kcal \cdot 2\ hr^{-1}$; $P = 0.005$). Heart rate ($P = 0.02$), systolic blood pressure ($P < 0.0001$), and RPP ($P = 0.002$) were higher for supplement compared to placebo.

Conclusion: Ingestion of OxyELITE Pro™ resulted in an increase in blood markers of lipolysis, as well as metabolic rate, during a two-hour post ingestion time period. An increase in hemodynamic variables was also observed. These findings are in reference to a sample of healthy men and women who were naïve to treatment with the dietary supplement. Additional work is needed to determine if the acute changes observed here would persist with chronic use of the supplement and possibly lead to weight/body fat loss over time.

Keyword: lipolysis, supplements, weight loss, thermogenesis

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Background

Obesity and overweight status has increased significantly in recent years, with an estimated 400 million individuals classified as obese,[1,2] and 1.6 billion classified as overweight.[2] While the optimal treatment plan for this epidemic likely includes increased physical activity coupled with modification and restriction in dietary intake, many individuals choose rather to focus on the use of pharmaceuticals or dietary supplements. In most industrialized nations, dietary supplements are big business. In fact, the supplement industry has been following an increasing sales trend since 2004 and was worth $61 billion to the United States economy in 2008, with an estimated 80 percent of adults purchasing supplements at least once per year.[3] Dietary supplements designed to aid in body weight/fat loss are one of the more popular classes of products sold, estimated to be a 700 million dollar industry in 2008.[4]

Unfortunately, most weight/fat loss supplements have little to no scientific support, in particular as pertaining to their use by human subjects. Moreover, some have been reported to cause ill-health.[5] As with many dietary supplements, those within the weight/fat loss category are often formulated based on results obtained from studies using isolated ingredients. While this alone is not problematic, the concern lies in the fact that most ingredients have only been studied in animals and not in human subjects, and the dosages used in such animal trials are often far greater than those used in finished products marketed to humans.

OxyELITE Pro™ is a dietary supplement marketed as a weight/fat loss aid. As with most dietary supplements, this product contains a combination of several ingredients purported to increase one or more aspects of metabolic rate or lipolysis, some of which have not yet been investigated in human subjects. Specifically, this product contains a proprietary blend of caffeine, bauhinia purpurea, bacopa monniera, geranium stem extract (1,3-dimethlyamylamine), cirsium oligophyllum, and rauwolscine extract as the active ingredients.

With the exception of caffeine, which has been investigated extensively in human subjects,[6,7] to our knowledge, little objective scientific evidence is available pertaining to the other ingredients as used by human subjects. It was our intention in the present study to investigate the acute effects of this supplement on blood markers of lipolysis, as well as metabolic rate. While we understand the need to conduct long-term intervention studies pertaining to the potential effect of this supplement on weight/fat loss over time, it was the purpose of the present study to first determine if the product resulted in an acute increase in our selected outcome measures. If so, then follow-up intervention studies would be warranted.

Methods

Subjects

Healthy, exercise-trained men (n = 6) and women (n=6) participated in this study. All subjects completed a medical history and physical activity questionnaire. No subject smoked cigarettes or had diagnosed disease of cardiovascular or metabolic origin. Men and women were very active and considered to be exercise-trained, as they performed combined aerobic and anaerobic exercise for an average of 7 and 5 hours per week, respectively, for the past several years. Subject descriptive characteristics are presented in Table 1. All experimental procedures were performed in accordance with the Helsinki Declaration. The University of Memphis Human Subjects Committee approved all experimental procedures. Subjects provided verbal and written consent prior to participating in this study.

Table 1. Characteristics of 6 men and 6 women.

Variable	Men	Women
Age (yrs)	24.8 ± 4.3	22.8 ± 0.4
Height (cm)*	179.3 ± 6.6	166.0 ± 9.0
Weight (kg)*	81.1 ± 11.6	62.3 ± 12.6
BMI ($kg \cdot m^{-2}$)	25.5 ± 5.0	22.4 ± 2.6
Body fat (%)*	14.7 ± 7.6	25.5 ± 3.1
Waist (cm)	84.6 ± 12.1	77.2 ± 6.8
Hip (cm)	102.6 ± 8.6	95.7 ± 6.6
Waist:hip	0.82 ± 0.05	0.81 ± 0.08
Years anaerobic exercise training	3.0 ± 3.6	2.2 ± 2.3
Hours per week anaerobic exercise	3.5 ± 2.7	1.6 ± 1.9
Years aerobic exercise training	1.6 ± 1.3	2.1 ± 1.6
Hours per week aerobic exercise	3.2 ± 2.0	3.4 ± 1.1

Notes: Data are mean ± SD. *Statistically significant difference noted in height ($P = 0.01$), weight ($P = 0.02$), and body fat ($P = 0.01$). No other statistically significant differences noted ($P > 0.05$).

Conditions and testing

Following screening procedures, subjects reported to the lab in the morning hours (0600–0900) on two different occasions separated by 3–4 days, to undergo testing. The time of day for testing was matched for each subject. Procedures described below were identical for both test sessions (supplement and placebo). The dietary supplement used in this investigation (OxyELITE Pro™; USPlabs, LLC; Dallas, TX) contained a proprietary blend of caffeine, bauhinia purpurea, bacopa monniera, geranium stem extract (1,3 dimethlyamlamine), cirsium oligophyllum, and rauwolscine extract as the active ingredients. Capsules were from the same bottle and produced in accordance with Good Manufacturing Practices. Subjects ingested two capsules of the dietary supplement, or an identical looking placebo (microcrystalline cellulose). The experiment was conducted as a double blind, crossover design. No other food was allowed during the two hour post intake period. However, water was allowed ad libitum, and was measured and matched for both days of testing (mean intake for men = 525 mL; mean intake for women = 317 mL).

Subjects reported to the laboratory in a fasted state (≥10 hours), without caffeine consumption during the past 10 hours. Subjects were asked not to exercise or to perform any strenuous physical activity for the 24 hours prior to each testing day. Following a 10 minute rest period, heart rate (via monitor) and blood pressure (via auscultation) were measured (rate pressure product was calculated as: HR × SBP = RPP), a blood sample was obtained, and subjects provided a five minute breath sample (for analysis of metabolic rate). Subjects then ingested either the supplement or placebo, in the presence of an investigator. At all other measurement times (30, 60, 90, and 120 minutes post ingestion), the same order of collection as described above was followed; however, blood was only collected at 60 and 120 minutes post ingestion. Subjects remained inactive in the laboratory during the entire two hour test period.

The measurement of metabolic rate was performed using indirect calorimetry via breath-by-breath collection (SensorMedics V_{max} 229 metabolic system; Yorba Linda, CA). All gas collection took place in a temperature and humidity controlled laboratory. The flow sensor and gas analyzers were calibrated prior to data collection each day. Total oxygen consumption ($L \cdot min^{-1}$) was determined from gas collection and total kilocalorie expenditure was estimated from this value. The respiratory exchange ratio (RER) was also determined from gas collection data (VCO_2/VO_2), and used as a measure of substrate utilization.

Blood collection and biochemistry

A total of three venous blood samples (7 mL per draw) were taken from subjects' forearm via needle and Vacutainer® (pre ingestion, 60, and 120 minutes post ingestion). Blood was immediately processed in a refrigerated centrifuge in order to obtain plasma (4 °C for 15 min at 2000 × g). Plasma samples were then stored in multiple aliquots at −70 °C. Assays were performed in duplicate on first thaw within four weeks of sample collection. Glycerol was determined using the Free Glycerol Determination Kit (FG0100) and Glycerol Standard (G7793), following the instructions of the manufacturer (Sigma Aldrich; St. Louis, MO). Free fatty acids (FFA) were determined using the Free Fatty Acid Quantification Kit (K612-100) following the instructions of the manufacturer (BioVision; Mountain View, CA).

Dietary intake

Subjects were required to record all food and drink consumed during the 24 hour period prior to the initial test day. Records were then copied and returned to subjects so that they could attempt to mimic this intake during the 24 hour period prior to the second test day. Records were analyzed for total calories, protein, carbohydrate, fat, and a variety of micronutrients (Food Processor SQL, version 9.9, ESHA Research, Salem, OR).

Statistical analysis

Area under the curve (AUC) was calculated for biochemical and metabolic data using the trapezoidal method (AUC_G) as described in detail by Pruessner et al[8] Statistical comparisons for biochemical (AUC_G) and metabolic data were made using a 2 (sex) × 2 (condition) repeated measures analysis of variance (RMANOVA). Biochemical, metabolic, and hemodynamic (% change) data were also compared using a sex × condition × time RMANOVA. Tukey's *post hoc* testing was used when needed. All analyses were performed using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC).

Statistical significance was set at $P \leq 0.05$. The data are presented as mean ± SEM, except for subject descriptive characteristics (mean ± SD).

Results

Subject characteristics, dietary data, and subjective response to supplement

Expected differences were noted between men and women for selected anthropometric variables (Table 1). The same was true of dietary intake of kilocalories ($P = 0.0006$), protein ($P < 0.0001$), carbohydrate ($P = 0.008$), and fat ($P = 0.008$), with men consuming more than women.

Most importantly, no differences were noted between conditions for total kilocalorie intake, protein, carbohydrates, fat, vitamin C, vitamin E, or vitamin A consumption during the 24 hours prior to each test day ($P > 0.05$). Dietary data are presented in Table 2. In terms of subjective response to treatment with the supplement, some subjects reported feeling "jittery", "on-edge", "sweaty", and "shaky", sometimes involving cold sweats, a racing heart beat, and poor sleep quality on the night of treatment.

Biochemical data

When considering the AUC analysis, no sex × condition interactions were noted for glycerol ($P = 0.53$) or FFA ($P = 0.08$). However, a condition ($P = 0.001$) and sex ($P = 0.0007$) effect was noted for glycerol, with values higher for supplement compared to placebo and higher for women compared to men. Data are presented in Figure 1. A condition ($P < 0.0001$), but not sex ($P = 0.85$) effect was noted for FFA, with values higher for supplement compared to placebo. Data are presented in Figure 2.

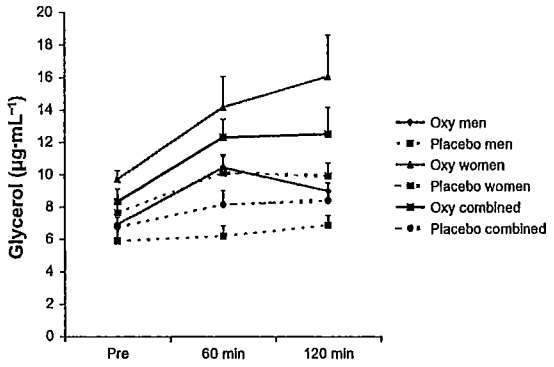

Figure 1. Plasma glycerol before and following ingestion of placebo and OxyELITE Pro™ by men and women.
Notes: Data are mean ± SEM. Sex × Condition × Time: $P = 0.43$; Sex × Time: $P = 0.25$; Condition × Time: $P = 0.21$; Sex × Condition: $P = 0.23$; Time: $P = 0.001$ (60 min and 120 min higher than Pre; $P < 0.05$); Condition: $P < 0.0001$; Sex: $P < 0.0001$; **Area under the curve.** Sex × Condition: $P = 0.53$; Condition: $P = 0.001$; Sex: $P = 0.0007$; OxyELITE Pro™ Men: 18.40 ± 0.77 µg·mL^{-1}·2 hr^{-1}; Placebo Men: 12.61 ± 1.12µg·mL^{-1}·2hr^{-1}; OxyELITE Pro™ Women: 27.07±3.01µg·mL^{-1}·2hr^{-1}; Placebo Women: 18.90 ± 1.73 µg·mL^{-1}·2 hr^{-1}; OxyELITE Pro™ Combined: 22.74 ± 1.98 µg·mL^{-1}·2 hr^{-1}; Placebo Combined: 15.76 ± 1.36 µg·mL^{-1}·2 hr^{-1}.

Metabolic data

When considering the AUC analysis for kilocalories, no sex × condition interactions were noted ($P = 0.26$). However, a condition ($P = 0.005$) and sex ($P = 0.002$) effect was noted, with values higher for supplement compared to placebo and higher for men compared to women. Data for kilocalories are presented in Figure 3 and data for RER are presented in Figure 4.

Hemodynamic data

Several differences were noted for both absolute and percent change data for hemodynamic variables. Absolute data are presented in Tables 3, 4, 5, and 6. Percent change data are presented in Figures 5 and 6.

Table 2. Dietary data of 6 men and 6 women during the 24 hour period before ingestion of placebo and OxyELITE Pro™.

Variable	Men		Women		Combined	
	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo
Kcal	2582 ± 376	2972 ± 353	1477 ± 245	1482 ± 277	2030 ± 271	2227 ± 310
Protein (g)	124 ± 18	131 ± 12	63 ± 7	63 ± 11	93 ± 13	97 ± 13
Carbohydrate (g)	368 ± 74	394 ± 69	207 ± 39	210 ± 43	287 ± 47	302 ± 48
Fat (g)	71 ± 9	98 ± 19	47 ± 10	47 ± 9	59 ± 7	73 ± 13
Vitamin C (mg)	126 ± 50	90 ± 37	45 ± 16	48 ± 11	85 ± 28	69 ± 19
Vitamin E (mg)	6 ± 4	8 ± 5	7 ± 3	7 ± 3	7 ± 2	8 ± 3
Vitamin A (RE)	638 ± 245	588 ± 246	380 ± 139	360 ± 142	509 ± 140	474 ± 140

Notes: Data are mean ± SEM. No statistically significant Sex × Condition or condition effects ($P > 0.05$). Sex effect for Kcal ($P = 0.0006$), protein ($P < 0.0001$), carbohydrate ($P = 0.008$), and fat ($P = 0.008$).

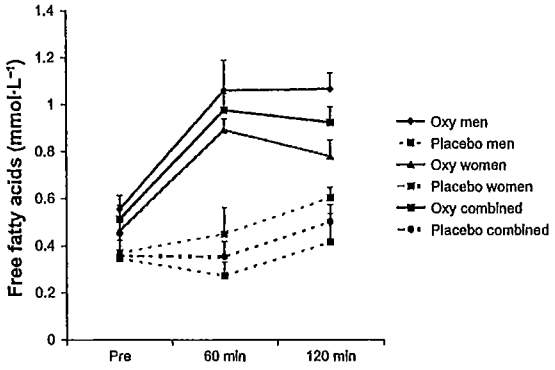

Figure 2. Plasma free fatty acids before and following ingestion of placebo and OxyELITE Pro™ by men and women.
Notes: Data are Mean ± SEM. Sex × Condition × Time: $P = 0.37$; Sex × Time: $P = 0.91$; Condition × Time: $P = 0.002$; Sex × Condition: $P = 0.004$; Time: $P < 0.0001$; Condition: $P < 0.0001$; Sex: $P = 0.62$; **Area under the curve.** Sex × Condition: $P = 0.08$; Condition: $P < 0.0001$; Sex: $P = 0.85$; OxyELITE Pro™ Men: 1.75 ± 0.11 mmol·L^{-1}·2 hr^{-1}; Placebo Men: 0.65 ± 0.16 mmol·L^{-1}·2 hr^{-1}; OxyELITE Pro™ Women: 1.51 ± 0.07 mmol·L^{-1}·2 hr^{-1}; Placebo Women: 0.94 ± 0.15 mmol·L^{-1}·2 hr^{-1}; OxyELITE Pro™ Combined: 1.62 ± 0.07 mmol·L^{-1}·2 hr^{-1}; Placebo Combined: 0.78 ± 0.12 mmol·L^{-1}·2 hr^{-1}.

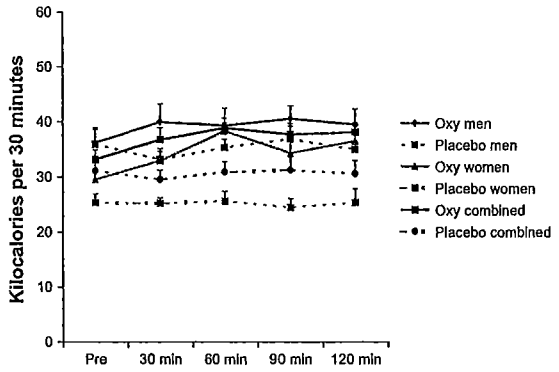

Figure 3. Kilocalorie expenditure before and following ingestion of placebo and OxyELITE Pro™ by men and women.
Notes: Data are mean ± SEM. Sex × Condition × Time: $P = 0.81$; Sex × Time: $P = 0.76$; Condition × Time: $P = 0.37$; Sex × Condition: $P = 0.01$; Time: $P = 0.41$; Condition: $P < 0.0001$; Sex: $P < 0.0001$; **Area under the curve.** Sex × Condition: $P = 0.26$; Condition: $P = 0.005$; Sex: $P = 0.002$; OxyELITE Pro™ Men: 158 ± 10 kcal·2 hr^{-1}; Placebo Men: 141 ± 8 kcal·2 hr^{-1}; OxyELITE Pro™ Women: 137 ± 3 kcal·2 hr^{-1}; Placebo Women: 101 ± 6 kcal·2 hr^{-1}; OxyELITE Pro™ Combined: 149 ± 7 kcal·2 hr^{-1}; Placebo Combined: 122 ± 8 kcal·2 hr^{-1}.

Discussion

Our data indicate that the dietary supplement OxyELITE Pro™, at a dosage of two capsules, results in an increase in plasma glycerol and FFA, as well as an increase in metabolic rate. This finding is observed in both men and women who are young and healthy. Future study may include a sample of older and/or overweight individuals to determine if similar results are observed.

Based on the findings for increased lipolysis and metabolic rate, it might be hypothesized that the supplement may aid in weight/fat loss over time. As stated earlier, this product contains a combination of caffeine, bauhinia purpurea, bacopa monniera, geranium stem extract (1,3-dimethlyamylamine), cirsium oligophyllum, and rauwolscine extract. Caffeine has lipolytic and thermogenic effects due to its ability to impair the degradation of cAMP as well as increase cAMP production via beta-adrenergic receptor independent and dependent mechanisms, respectively.[9] The independent effects appear due to the ability of caffeine to directly inhibit cAMP degradation, by inhibiting the cyclic nucleotide phosphodiesterase[10] and blocking adenosine receptors. The direct effect results from an increase in catecholamine release following caffeine ingestion, which may be secondary to the previously described adenosine inhibition.[9]

Findings of increased metabolic rate and circulating markers of fatty acid degradation have been reported previously for caffeine alone.[11–13] Depending on the dosage of caffeine used, our findings generally appear similar or greater in magnitude, highlighting the potential influence of the other ingredients contained within this supplement. That being said, little is known regarding the other active ingredients, at least as pertaining to use by human subjects. For example, bauhinia purpurea has been reported to have thyroid stimulating properties, with no signs of overt toxicity.[14,15] Thyroid hormones play an important

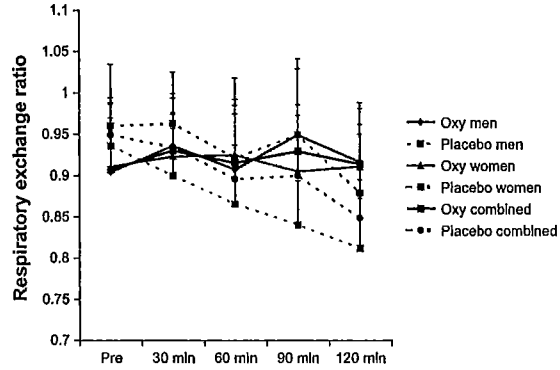

Figure 4. Respiratory exchange ratio before and following ingestion of placebo and OxyELITE Pro™ by men and women.
Notes: Data are mean ± SEM. Sex × Condition × Time: $P = 1.00$; Sex × Time: $P = 0.97$; Condition × Time: $P = 0.86$; Sex × Condition: $P = 0.38$; Time: $P = 0.85$; Condition: $P = 0.61$; Sex: $P = 0.27$.

Table 3. Heart rate (bpm) before and following ingestion of placebo and OxyELITE Pro™ by men and women.

Time	Men		Women		Combined	
	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo
Pre	60 ± 2	67 ± 3	72 ± 5	68 ± 4	66 ± 3	68 ± 2
30 min	55 ± 3	61 ± 3	73 ± 10	63 ± 4	64 ± 6	62 ± 2
60 min	59 ± 3	61 ± 3	79 ± 10	61 ± 4	69 ± 6	61 ± 2
90 min	61 ± 3	59 ± 2	72 ± 7	60 ± 3	67 ± 4	59 ± 2
120 min	62 ± 3	58 ± 3	79 ± 10	61 ± 4	70 ± 5	59 ± 2

Notes: Data are mean ± SEM. Sex × Condition × Time: $P = 0.96$; Sex × Time: $P = 0.97$; Condition × Time: $P = 0.37$; Sex × Condition: $P = 0.002$; Time: $P = 0.77$; Condition: $P = 0.02$; Sex: $P = 0.0003$.

Table 4. Systolic blood pressure (mmHg) before and following ingestion of placebo and OxyELITE Pro™ by men and women.

Time	Men		Women		Combined	
	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo
Pre	105 ± 3	105 ± 4	102 ± 4	97 ± 3	103 ± 2	101 ± 3
30 min	111 ± 4	106 ± 3	113 ± 9	95 ± 2	112 ± 5	101 ± 2
60 min	120 ± 5	108 ± 3	112 ± 6	97 ± 2	116 ± 4	102 ± 3
90 min	125 ± 3	109 ± 6	111 ± 5	99 ± 2	118 ± 3	104 ± 3
120 min	123 ± 4	109 ± 4	114 ± 4	96 ± 3	118 ± 3	102 ± 3

Notes: Data are mean ± SEM. Sex × Condition × Time: $P = 0.70$; Sex × Tme: $P = 0.62$; Condition × Time: $P = 0.16$; Sex × Condition: $P = 0.22$; Time: $P = 0.02$ (90 min and 120 min higher than pre; $P < 0.05$); Condition: $P < 0.0001$; Sex: $P < 0.0001$.

Table 5. Diastolic blood pressure (mmHg) before and following ingestion of placebo and OxyELITE Pro™ by men and women.

Time	Men		Women		Combined	
	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo
Pre	61 ± 4	62 ± 2	59 ± 3	60 ± 1	60 ± 2	61 ± 1
30 min	71 ± 3	67 ± 4	66 ± 4	59 ± 2	68 ± 2	63 ± 3
60 min	67 ± 4	66 ± 4	59 ± 3	56 ± 2	63 ± 3	61 ± 3
90 min	64 ± 6	65 ± 4	60 ± 4	55 ± 3	62 ± 4	60 ± 3
120 min	66 ± 3	69 ± 4	67 ± 2	58 ± 3	67 ± 2	64 ± 3

Notes: Data are mean ± SEM. Sex × Condition × Time: $P = 0.72$; Sex × Time: $P = 0.68$; Condition × Time: $P = 0.73$; Sex × Condition: $P = 0.14$; Time: $P = 0.14$; Condition: $P = 0.21$; Sex: $P = 0.0002$.

Table 6. Rate pressure product before and following ingestion of placebo and OxyELITE Pro™ by men and women.

Time	Men		Women		Combined	
	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo	OxyELITE Pro™	Placebo
Pre	6258 ± 348	7163 ± 508	7304 ± 553	6594 ± 402	6781 ± 349	6878 ± 320
30 min	6083 ± 421	6514 ± 424	8615 ± 1997	5991 ± 441	7349 ± 1045	6252 ± 302
60 min	7132 ± 541	6604 ± 367	9035 ± 1579	5897 ± 403	8084 ± 846	6251 ± 281
90 min	7661 ± 516	6421 ± 551	7947 ± 700	5926 ± 288	7808 ± 417	6174 ± 306
120 min	7603 ± 453	6318 ± 437	8993 ± 1177	5848 ± 422	8298 ± 637	6083 ± 298

Notes: Data are mean ± SEM. Sex × Condition × Time: $P = 0.85$; Sex × Time: $P = 0.88$; Condition × Time: $P = 0.24$; Sex × Condition: $P = 0.004$; Time: $P = 0.92$; Condition: $P = 0.002$; Sex: $P = 0.20$.

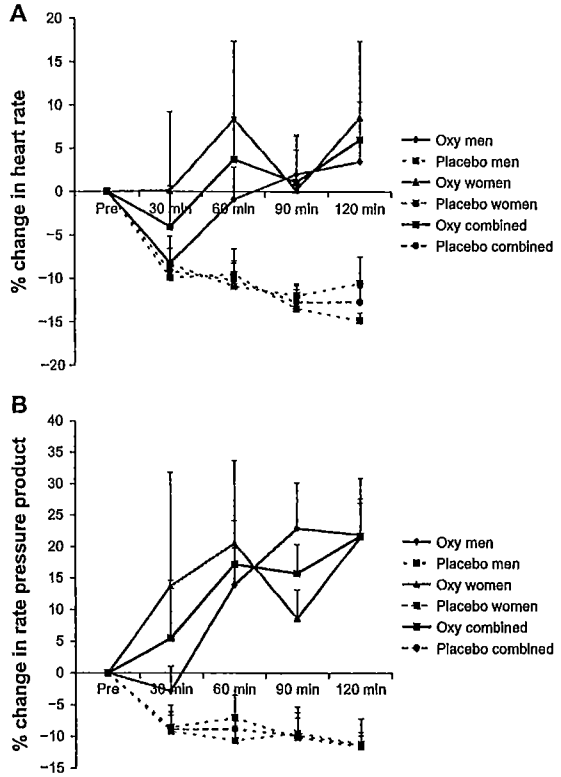

Figure 5. Percent change from pre in heart rate (A) and rate pressure product (B) for placebo and OxyELITE Pro™.
Notes: Data are mean ± SEM. **Heart rate.** Sex × Condition × Time: $P = 0.78$; Sex × Time: $P = 0.88$; Condition × Time: $P = 0.02$; Sex × Condition: $P = 0.44$; Time: $P = 0.31$; Condition: $P < 0.0001$; Sex: $P = 0.22$; **Rate pressure product.** Sex × Condition × Time: $P = 0.40$; Sex × Time: $P = 0.66$; Condition × Time: $P = 0.006$; Sex × Condition: $P = 0.71$; Time: $P = 0.58$; Condition: $P < 0.0001$; Sex: $P = 0.98$.

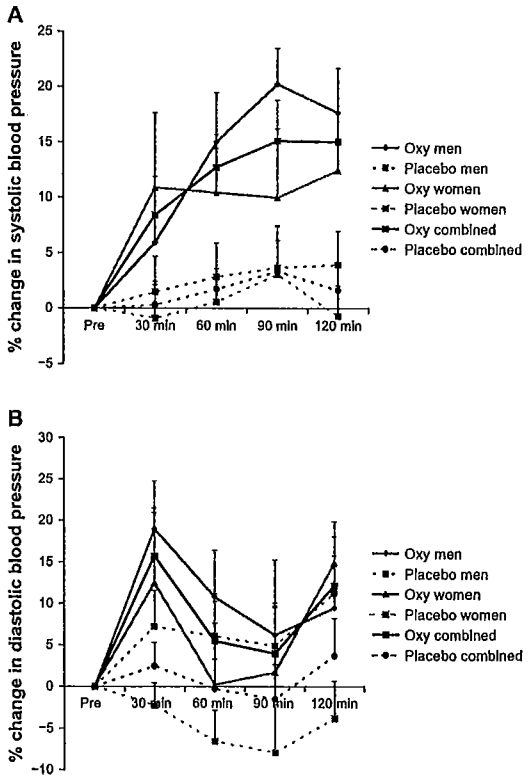

Figure 6. Percent change from pre in systolic (A) and diastolic (B) blood pressure for placebo and OxyELITE Pro™.
Notes: Data are mean ± SEM. **Systolic blood pressure.** Sex × Condition × Time: $p = 0.50$; Sex × Time: $P = 0.58$; Condition × Time: $P = 0.07$; Sex × Condition: $P = 0.70$; Time: $P = 0.003$; Condition: $P < 0.0001$; sex: $P = 0.10$; **Diastolic blood pressure.** Sex × Condition × Time: $P = 0.69$; Sex × Time: $P = 0.44$; Condition × Time: $P = 0.52$; Sex × Condition: $P = 0.22$; Time: $P = 0.02$; Condition: $P = 0.006$; Sex: $P = 0.002$.

role in metabolic rate, with an increase in thyroid function possibly leading to an increase in energy expenditure. Bacopa monniera has also been reported to increase thyroid hormone.[16] and clinical trials have not found any adverse effects of bacopa monniera when consumed at a dosage of 300 mg · day^{-1},[17] or as a component of a dietary supplement (BacoMind™) delivered at 300–450 mg · day^{-1}.[18]

The action of 1,3-dimethlyamylamine appears as a simple aliphatic amine, functioning as a norepinephrine reuptake inhibitor and/or norepinephrine releasing agent, which may help explain the increase in circulating free fatty acids and glycerol with supplement ingestion. Anecdotal reports indicate a potential effect on appetite suppression and suggestions of increased weight/fat loss. However, to our knowledge, no published reports are available pertaining to these effects in human subjects.

Pharmacological studies of cirsium oligophyllum reveal that the active constituent possesses β-adrenergic receptor agonist activity and stimulates lipolysis in subcutaneous fat cells,[19] possibly due to the uncoupling of protein in vitro. Uncoupling proteins are known to be involved with thermogenesis and energy dissipation. Hence, this may help explain our finding of increased energy expenditure.

Finally, rauwolscine is a stereoisomer of yohimbine and functions as an α-adrenergic receptor antagonist. While rauwolscine is thought to have similar potency as yohimbine at the α_2-receptor, which allows for lipolysis, it appears to be approximately 50-fold less active at the α_1-receptor,[20] which inhibits lipolysis.

We are unaware of data pertaining to the efficacy and safety of rauwolscine use in human subjects.

Aside from the effects on metabolic rate and lipolysis, hemodynamic variables were altered in such as way as to indicate increased myocardial work. Specifically, heart rate and blood pressure were increased in response to treatment, with the obvious increase in the calculated rate pressure product. Although such changes are common with weight loss supplements,[21–27] in particular for those including caffeine and other stimulants, some caution should be advised when using this and similar products. This is particularly true for individuals who are hypertensive (resting blood pressure ≥140/90 mmHg) or for those who are pre-hypertensive (resting blood pressure ≥120/80 mmHg). For these individuals, it would be best to attempt weight/fat loss through both increased energy expenditure and modified/restricted dietary intake. As with the use of any weight/fat loss aid, it is prudent for individuals to be monitored by a qualified health care professional during the course of use.

Conclusion

In conclusion, we report that the product OxyELITE Pro™ ingested at a dosage of two capsules by young and healthy men and women, resulted in an increase in plasma glycerol and FFA, in addition to metabolic rate. These results are apparent along with an increase in heart rate and blood pressure. These latter findings (increased systolic blood pressure in particular) may warrant caution, in particular in those with pre-hypertension or hypertension. The use of a lower dosage may attenuate this response. While this study only provides acute data pertaining to the lipolytic and thermogenic effects of this supplement, well-controlled intervention trials are needed in order to determine the chronic effects of the supplement on body weight/fat loss and associated metabolic and biochemical markers of health, in particular within a sample of overweight or obese subjects.

Acknowledgments

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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

CGM, TMF, REC, and RJA 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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Nutrition and Metabolic Insights

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Biochemical and Anthropometric Effects of a Weight Loss Dietary Supplement in Healthy Men and Women

Cameron G. McCarthy, Robert E. Canale, Rick J. Alleman Jr., Jacob P. Reed and Richard J. Bloomer

Cardiorespiratory/Metabolic Laboratory, The University of Memphis, Memphis, TN 38152, USA.
Corresponding author email: rbloomer@memphis.edu

Abstract

Background: We have recently noted an acute increase in circulating free fatty acids and glycerol, as well as resting metabolic rate, when men and women ingested the dietary supplement OxyELITE Pro™ in a single dose. We have also noted a reduction in appetite when subjects were treated with this supplement for 14 consecutive days. It is possible that such findings may favor body weight and fat loss over time. Therefore, the purpose of the present study was to determine the effects of this dietary supplement on weight loss and associated markers using an eight week intervention.

Methods: Exercise-trained subjects were randomly assigned in double blind manner to ingest either the dietary supplement (n = 16; aged 22.8 ± 0.7) or a placebo (n = 16; 22.5 ± 0.5) every day for eight weeks. Body weight, body composition, skinfold thickness, serum lipids, and appetite were measured as the primary outcome variables. As measures of supplement safety, a complete blood count and comprehensive metabolic panel were performed, and resting heart rate and blood pressure were measured (pre and post intervention).

Results: No interactions or main effects were noted for our primary outcome measures ($P > 0.05$). However, when comparing pre and post intervention values for the supplement, significant decreases were noted in appetite, body weight, body fat percentage, and skinfold thickness ($P < 0.05$), while increases were noted for total and HDL-C, as well as for resting heart rate ($P < 0.05$). No changes were noted for placebo from pre to post intervention ($P > 0.05$), with the exception of an increase in HDL-C ($P < 0.05$). Blood pressure and bloodborne safety variables were not differently impacted by supplement or placebo ($P > 0.05$), with the exception of monocytes, for which an interaction effect was noted ($P = 0.04$).

Conclusion: These data indicate that the dietary supplement OxyELITE Pro™ may assist in weight and body fat loss in a sample of exercise-trained men and women. The supplement does not result in any adverse effects pertaining to resting blood pressure or blood-borne markers of safety; however a small increase in resting heart rate is observed.

Keywords: supplement, adiposity, body fat, lipids

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Background

The prevalence of obesity and overweight status has increased to epidemic proportions within recent years, with approximately 400 million individuals classified as obese,[1,2] and 1.6 billion classified as overweight.[2] While the ideal treatment plan for this problem includes increased physical activity[3–5] and modification and restriction of dietary intake,[6] additional options exist. These include the use of pharmaceuticals[7] and/or surgical intervention,[8] as well as the use of dietary supplements.[9]

With regards to the latter, dietary supplements continue to increase in popularity, with 80 percent of adults purchasing dietary supplements at least once per year,[10] with an estimated worth of $61 billion to the United States economy in 2008. One class of supplement that appears in high demand is designed to aid in body weight and fat loss.[11] These products have the potential not only to aid in reducing body weight and fat, but also to improve the serum lipid panel.

We have recently studied the effects of the weight loss dietary supplement OxyELITE Pro™, using both an acute laboratory study focused on metabolic rate and blood markers of lipolysis,[12] as well as a 14 day intervention study focused on appetite suppression and clinical safety markers (eg, complete blood count, metabolic panel, lipid panel).[13] Both of these studies involved young and healthy subjects. The supplement has been noted to result in an increase in circulating free fatty acids and glycerol, in addition to an increase in resting metabolic rate (when delivered as a single dose). A slight reduction in appetite has also been observed. No adverse effects have been noted with regards to clinical safety markers (eg, blood chemistry). Despite these findings, no scientific evidence is currently available pertaining to the effects of this dietary supplement following a longer term intervention period. This is true with regards to anthropometric and safety data, as well as other bloodborne variables of interest associated with obesity such as serum lipids and markers of oxidative stress (eg, malondialdehyde). Therefore, the purpose of the present study was to determine the effects of OxyELITE Pro™ on weight loss and associated markers following an eight week intervention, using a randomized, placebo controlled, double blind design. We hypothesized that subjects in the supplement group would experience more favorable changes in weight loss and associated parameters compared to subjects in the placebo group.

Methods and Procedures

Subjects

Thirty-two recreationally exercise-trained men or women (5.2 ± 0.3 hours per week of exercise; 3.9 ± 0.5 years of exercise) between the ages of 19 and 36 years participated. The indicated sample of 32 subjects is similar to many other studies involving weight loss dietary supplements. Our inclusion of men and women of younger age was done in an attempt to mimic the subject population which represents the market for weight loss dietary supplements. Traditionally, weight loss studies include only young to middle aged obese subjects, typically women who are inactive. One unique aspect of our study is the inclusion of an equal number of men and women, all who exercise regularly, and most who are not obese. Such a sample has greater generalizability to the target market for weight loss supplements. Subjects were nonsmokers and did not have any cardiovascular or metabolic problems that might affect their response to treatment and their ability to participate. Health history, drug and dietary supplement usage, and physical activity questionnaires were completed by all subjects to determine eligibility.

Concerning human subjects, each was informed of all procedures, potential risks, and benefits associated with the study through both verbal and written form prior to participation. This was done in accordance with the Declaration of Helsinki and the procedures approved by the University Institutional Review Board for Human Subjects Research (H11-24). All subjects signed an informed consent form prior be being admitted.

Screening

During the initial visit to the laboratory, subjects completed the informed consent form, health and physical activity questionnaires. Subjects were provided with food logs and instructions regarding how to complete these logs during the week prior to beginning their assigned condition and during the final week of the assigned condition (please see description below). Subjects received a detailed schedule for the entire study period outlining all pertinent dates of participation.

Testing

For both visits to the laboratory (pre and post intervention), subjects reported in the morning hours (5:00–11:00 am) following a 10 hour overnight fast. Upon arrival, food records were collected and reviewed with subjects. Subjects were then asked to void. Subjects then sat in a chair and rested for 10 minutes. Heart rate (via 60 second palpation of the radial artery) and blood pressure (via auscultation using a two-earpiece stethoscope) was then measured and recorded. A blood sample was then obtained (described below). Subjects were then asked to record their overall appetite during the prior two weeks, using a 10 point visual analog where the scale was anchored with "0 = none at all and 10 = extreme." Following this, subjects' height, weight, waist and hip circumference, skinfold thickness (7 site using a Lange caliper), and body composition was measured. Body composition was determined by dual energy x-ray absorptiometry (DEXA; Hologic QDR-4500W) using a 6-minute fan array. Both total and regional (trunk specific) body fat were determined, and fat and fat free mass were calculated. The DEXA assessment was performed by a licensed technician. These exact procedures were followed for both test days (pre and post intervention).

Blood collection and biochemistry

Blood was collected from subjects on two different days throughout the course of the study: pre intervention (day 1 of the study—the morning of the first day of supplement or placebo use) and post intervention (the day following the final day of supplementation). On each occasion, venous blood samples (~25 mL) were taken from subjects via needle and Vacutainer®. All blood samples were collected in a fasted and 10 minute rested state. Following collection, samples were processed and immediately placed in the refrigerator or the freezer, depending on the sample. A portion of blood samples were sent to Laboratory Corporation of America for analysis of lipid panel, complete blood count, and comprehensive metabolic panel. The lipid panel was determined using enzymatic procedures (Roche/Hitachi Modular). The complete blood count was determined using an automated cell counter (Coulter LH750). The comprehensive metabolic panel was determined using automated procedures (Roche/Hitachi Modular). Remaining blood was stored at –70 °C until analyzed for malondialdehyde, following the procedures of Jentzsch et al,[14] using reagents purchased from Northwest Life Science Specialties (Vancouver, WA).

Supplementation

Subjects were randomly assigned (via coin flip by an investigator not involved in data collection) in double blind manner to consume either a placebo (n = 16; 8 men and 8 women) or the supplement (OxyELITE Pro™; USPlabs, LLC, Dallas, TX; n = 16; 8 men and 8 women). None of the investigators involved in this work have a financial interest in USPlabs, LLC. The supplement contained a proprietary blend of caffeine, bauhinia purpurea, bacopa monniera, geranium stem extract (1,3 dimethlyamlamine), cirsium oligophyllum, and rauwolscine extract as the active ingredients. Capsules were produced in accordance with Good Manufacturing Practices and from the same lot number. The placebo (microcrystalline cellulose) and OxyELITE Pro™ capsules were identical in appearance and were dispensed to subjects in identically labeled bottles, at the start of the study and after four weeks. Neither the subjects nor the investigators involved in data collection were aware of the assigned treatment.

Subjects were instructed to use the supplement or placebo in the same manner as suggested on the product label. Specifically, subjects were instructed to "Ingest 1 capsule daily for the first three days. If the single capsule each day is well-tolerated, then, starting on day four, try ingesting an additional capsule, 5–6 hours after the first capsule. If this is well-tolerated, then this will be your dosage throughout the eight week study period. If, however, taking the second capsule causes any adverse effects such as sleeplessness, then you should attempt to ingest 2 capsules at once in the morning, provided that it is well-tolerated. If ingesting 2 capsules at once in the morning is not well-tolerated, then you should revert back to 1 capsule daily in the morning." Therefore, subjects were provided the option to use either 1 or 2 capsules per day. This was done in an attempt to duplicate the conditions in which individuals would use this dietary supplement in a non-laboratory based setting. Subjects reported their intake to investigators (when capsule bottles were returned) and compliance to treatment was determined by counting remaining capsules. For both conditions, capsules were taken with water on an

empty stomach in the early morning, and if taking a second dosage, during the early-mid afternoon.

Dietary records and physical activity

Subjects were instructed to maintain their normal diet (inclusive of food and beverages) during the eight week study period and to record intake during the week prior to each test day. Diet records were analyzed for total kilocalories, protein, carbohydrate, fat, and a variety of other nutrients (Food Processor SQL, version 9.9, ESHA Research, Salem, OR). Subjects were asked to maintain their normal physical activity habits and exercise training schedule during the study period, with the exception of the two days (48 hours) prior to each test day, in which they were asked not to perform any strenuous exercise. Subjects were not required to maintain activity logs.

Statistical analysis

Outcome measures were analyzed using a 2 (condition) × 2 (pre/post intervention) analysis of variance (ANOVA). The primary outcome measures were also analyzed using a paired *t*-test (comparing pre and post intervention data independently for both supplement and placebo). The data are presented as mean ± SEM. All analyses were performed using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC). Statistical significance was set at $P \leq 0.05$.

Results

Subject compliance to treatment, appetite, descriptive characteristics, anthropometric and hemodynamic data are presented in Table 1. All subjects assigned to the placebo ingested two capsules per day. Of the 16 subjects assigned to the supplement, 11 ingested two capsules per day and five ingested only one capsule per day. These five subjects indicated that the ingestion of two capsules was associated with increased feelings of jitters and sleeplessness. None of the remaining 11 subjects assigned to the supplement noted any adverse effects of treatment. Due to scheduling conflicts, four subjects (three using supplement;

Table 1. Descriptive characteristics, anthropometric and hemodynamic data for 32 men and women assigned to OxyELITE Pro™ or placebo for eight weeks.

Variable	OxyELITE Pro™			Placebo		
	Pre (n = 16)	Post (n = 16)	*P* value**	Pre (n = 16)	Post (n = 16)	*P* value**
Compliance to treatment (%)	NA	93.0 ± 1.2	NA	NA	94.6 ± 1.9	NA
Age (years)	22.8 ± 1.0	NA	NA	22.5 ± 0.7	NA	NA
Height (cm)	172.9 ± 2.6	NA	NA	170.3 ± 1.3	NA	NA
Body weight (kg)	78.0 ± 4.1	76.1 ± 3.9	<0.01	75.2 ± 2.4	74.6 ± 2.5	0.15
BMI ($kg \cdot m^{-2}$)	26.0 ± 1.2	25.4 ± 1.1	<0.01	25.9 ± 0.7	25.7 ± 0.7	0.14
Waist (cm)	83.8 ± 3.7	81.2 ± 3.3	<0.01	81.2 ± 2.0	80.5 ± 2.2	0.14
Hip (cm)	103.4 ± 2.0	102.3 ± 1.6	0.10	101.8 ± 1.4	101.7 ± 1.4	0.96
Waist:Hip	0.81 ± 0.02	0.79 ± 0.02	<0.01	0.80 ± 0.02	0.79 ± 0.02	0.14
DEXA total body fat (%)	24.1 ± 2.1	23.1 ± 2.0	0.02	25.1 ± 2.3	25.0 ± 2.3	0.87
DEXA trunk body fat (%)	22.6 ± 2.4	21.8 ± 2.3	0.09	24.3 ± 2.2	24.0 ± 2.2	0.57
Total fat mass (kg)	18.5 ± 1.7	17.3 ± 1.6	<0.01	18.7 ± 1.8	18.5 ± 1.8	0.51
Total fat free mass (kg)	59.5 ± 3.8	58.8 ± 3.7	0.03	56.5 ± 2.7	56.1 ± 2.7	0.14
Skinfold thickness (mm)	147.3 ± 16.2	130.4 ± 14.9	<0.01	144.8 ± 15.4	138.0 ± 14.8	0.06
Heart rate (bpm)	63.3 ± 1.9	69.4 ± 2.2	<0.01	65.1 ± 2.1	66.9 ± 2.9	0.35
Systolic blood pressure (mmHg)	114.1 ± 3.2	117.2 ± 3.5	0.35	112.3 ± 2.3	111.8 ± 2.6	0.97
Diastolic blood pressure (mmHg)	70.1 ± 3.2	72.9 ± 2.1	0.29	70.7 ± 2.6	70.0 ± 2.6	0.88

Notes: Values are mean ± SEM. No condition × Pre/Post intervention interaction effects noted ($P > 0.05$). No condition effects noted ($P > 0.05$). No pre/post intervention effects noted ($P > 0.05$). ***P* values obtained using paired *t*-tests for each condition.

one using placebo) had their post intervention DEXA scan performed one day prior to the end of the 8 week intervention (after 55 days of treatment).

As can be viewed in Table 1, compliance to both the placebo and the supplement was excellent and not different between conditions ($P = 0.49$). No interactions or main effects were noted for any anthropometric or hemodynamic variable ($P > 0.05$). However, when comparing pre and post intervention values for the supplement, significant decreases were noted in body weight, BMI, waist circumference, waist:hip, total body fat percentage, fat mass, fat free mass, and skinfold thickness ($P < 0.05$), while an increase was noted in resting heart rate ($P < 0.01$). No changes were noted for placebo from pre to post intervention ($P > 0.05$).

Lipid panel and malondialdehyde data are presented in Table 2. With the exception of malondialdehyde, no interactions or main effects were noted for any lipid specific parameter ($P > 0.05$). A condition ($P < 0.0001$) and pre/post intervention ($P = 0.02$) effect was noted for malondialdehyde, with values lower for supplement compared to placebo and at pre intervention compared to post intervention, respectively. When comparing pre and post intervention values for the supplement, significant increases were noted in total cholesterol, HDL-C, and malondialdehyde ($P < 0.05$), while a decrease was noted in LDL-C:HDL-C and total cholesterol:HDL-C ($P < 0.05$). For placebo, significant increases were noted in HDL-C and malondialdehyde ($P < 0.05$), while a decrease was noted in LDL-C:HDL-C and total cholesterol:HDL-C ($P < 0.05$).

Complete blood count data are presented in Table 3. An interaction effect was noted for monocytes ($P = 0.04$). A condition effect was noted for MCH ($P = 0.01$), MCHC ($P = 0.03$), and neutrophils ($P = 0.02$). A pre/post intervention effect was noted for RBC ($P = 0.01$), MCV ($P < 0.0001$), MCH ($P = 0.002$), MCHC ($P = 0.001$), platelets ($P = 0.002$), neutrophils ($P = 0.01$), and monocytes ($P = 0.002$). No other interactions or main effects were noted for complete blood count data ($P > 0.05$).

Metabolic panel data are presented in Table 4. A condition effect was noted for glucose ($P = 0.02$), potassium ($P = 0.05$), and alkaline phosphatase ($P = 0.01$). A pre/post intervention effect was noted for CO_2 ($P = 0.0002$). No other interactions or main effects were noted for metabolic panel data ($P > 0.05$).

Appetite and dietary intake data are presented in Table 5. A pre/post intervention effect was noted for appetite ($P = 0.01$), with values lower post intervention compared to pre intervention. This was mostly influenced by the supplement condition; appetite was lower from pre to post intervention for supplement ($P = 0.0006$) but not for placebo ($P > 0.05$). No other interactions or main effects were noted for dietary data ($P > 0.05$).

Discussion

The findings from our investigation indicate that the dietary supplement OxyELITE Pro™ may assist in weight and body fat loss, while improving selected markers of the blood lipid panel. At a daily dosage of

Table 2. Lipid specific data for 32 men and women assigned to OxyELITE Pro™ or placebo for eight weeks.

Variable	OxyELITE Pro™			Placebo		
	Pre (n = 16)	Post (n = 16)	Pro™ *P* value**	Pre (n = 16)	Post (n = 16)	*P* value**
Cholesterol ($mg \cdot dL^{-1}$)	147.6 ± 5.0	156.0 ± 4.7	0.05	153.9 ± 4.3	158.4 ± 3.4	0.49
Triglycerides ($mg \cdot dL^{-1}$)	77.1 ± 9.3	62.6 ± 8.1	0.07	81.3 ± 8.1	87.3 ± 10.8	0.39
HDL-C ($mg \cdot dL^{-1}$)	50.0 ± 2.9	58.6 ± 3.7	<0.01	54.4 ± 3.0	58.3 ± 3.8	0.03
VLDL-C ($mg \cdot dL^{-1}$)	15.4 ± 1.8	12.6 ± 1.6	0.08	16.3 ± 1.6	17.5 ± 2.2	0.41
LDL-C ($mg \cdot dL^{-1}$)	82.6 ± 4.4	84.8 ± 4.1	0.50	83.2 ± 5.0	82.6 ± 3.3	0.44
LDL-C/HDL-C	1.8 ± 0.2	1.6 ± 0.2	0.04	1.6 ± 0.2	1.5 ± 0.1	<0.01
Total:HDL-C	3.1 ± 0.2	2.8 ± 0.2	<0.01	3.0 ± 0.2	2.9 ± 0.2	0.03
Malondialdehyde ($\mu mol \cdot L^{-1}$)*,†	0.52 ± 0.04	0.58 ± 0.04	0.04	0.85 ± 0.05	1.32 ± 0.20	0.02

Notes: Values are mean ± SEM. No condition x Pre/Post intervention interaction effects noted ($P > 0.05$); trend noted for malondialdehyde ($P = 0.07$). *Condition effect noted for malondialdehyde ($P < 0.0001$). No other condition effects noted ($P > 0.05$); †Pre/Post intervention effect noted for malondialdehyde ($P = 0.02$). No pre/post intervention effects noted ($P > 0.05$); **P values obtained using paired t-tests for each condition.

Table 3. Complete blood count data for 32 men and women assigned to OxyELITE Pro™ or placebo for eight weeks.

Variable	OxyELITE Pro™		Placebo	
	Pre (n = 16)	Post (n = 16)	Pre (n = 16)	Post (n = 16)
WBC ($10^3 \cdot \mu L^{-1}$)	6.4 ± 0.3	5.9 ± 0.5	6.2 ± 0.7	6.2 ± 0.4
RBC ($10^6 \cdot \mu L^{-1}$)†	4.7 ± 0.1	4.9 ± 0.1	4.5 ± 0.1	4.8 ± 0.1
Hemoglobin ($g \cdot dL^{-1}$)	14.1 ± 0.2	14.1 ± 0.3	14.1 ± 0.3	14.3 ± 0.3
Hematocrit (%)	41.9 ± 0.7	41.1 ± 1.0	41.4 ± 0.7	41.3 ± 0.9
MCV (fL)†	89.9 ± 1.2	84.3 ± 1.1	91.9 ± 1.2	86.6 ± 1.1
MCH (pg)*,†	30.3 ± 0.4	28.9 ± 0.5	31.3 ± 0.4	30.0 ± 0.4
MCHC ($g \cdot dL^{-1}$)*,†	33.6 ± 0.2	34.2 ± 0.1	34.0 ± 0.1	34.6 ± 0.2
RDW (%)	13.5 ± 0.3	13.2 ± 0.2	13.3 ± 0.2	13.0 ± 0.2
Platelets ($10^3 \cdot \mu L^{-1}$)†	183.8 ± 11.3	226.4 ± 17.8	208.4 ± 8.2	243.3 ± 8.0
Neutrophils (%)*,†	57.1 ± 2.5	48.8 ± 2.1	53.7 ± 2.9	49.9 ± 1.5
Lymphocytes (%)	33.2 ± 2.4	37.9 ± 2.3	34.8 ± 2.6	37.5 ± 1.6
Monocytes (%)**,†	6.5 ± 0.4	9.1 ± 0.6	8.1 ± 0.5	8.7 ± 0.4
Eosinophils (%)	2.9 ± 0.4	3.5 ± 0.6	2.9 ± 0.4	3.3 ± 0.6
Basophils (%)	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1

Notes: Values are mean ± SEM. *Condition effect noted for MCH ($P = 0.01$), MCHC ($P = 0.03$), and neutrophils ($P = 0.02$); trend noted for MCV ($P = 0.07$) and platelets ($P = 0.09$). No other condition effects noted ($P > 0.05$); **Condition × Pre/Post interaction effect noted for monocytes ($P = 0.04$). No other condition × Pre/Post intervention interaction effects noted ($P > 0.05$); †Pre/Post intervention effect noted for RBC ($P = 0.01$), MCV ($P < 0.0001$), MCH ($P = 0.002$), MCHC ($P = 0.001$), platelets ($P = 0.002$), neutrophils ($P = 0.01$), and monocytes ($P = 0.002$). No other pre/post intervention effects noted ($P > 0.05$).

one to two capsules, the supplement does not result in any adverse effects pertaining to bloodborne markers of safety (eg, liver function). However, the supplement does result in an increase in resting heart rate of approximately six beats per minute. Although this increase in heart rate was not accompanied by a significant increase in systolic or diastolic blood pressure (~3 mmHg), it may be wise for hypertensive individuals to avoid use of this supplement, as any increase in these variables may be undesirable.

These data extend our prior work with this supplement. Using an acute laboratory study involving single ingestion of the supplement or placebo in a crossover design, we noted an increase in circulating free fatty acids and glycerol, as well as an increase in resting metabolic rate in men and women.[12] Using an open label design, we have also noted a reduction in appetite when subjects were treated with this supplement each day for 14 consecutive days.[13] Based on these results, we hypothesized that chronic use of the supplement might decrease appetite and overall food intake, while also stimulate metabolic rate and lipolysis—equating to body weight and fat reduction over time. Our collective findings indicate this to be the case; that is, when comparing pre and post intervention data for subjects in the supplement condition.

From an efficacy point of view, the variables with the greatest interest in this investigation are those presented in Table 1. In terms of anthropometric variables, although no interaction effects were noted, when values were compared for each condition independently from pre to post intervention, we noted significant changes in many variables. For example, body weight, waist circumference, skinfold thickness, and body fat percentage were decreased. However, it should be noted that a small portion of the body weight lost was fat free mass, a common finding in weight loss intervention studies.[15,16]

In terms of blood lipids, although no interaction effects were noted, when values were compared for each condition independently from pre to post intervention, we noted significant changes in many variables. First, malondialdehyde was increased from pre to post intervention in both conditions. This was surprising, as lipid peroxidation and oxidative stress are associated with levels of adiposity and an obese state.[17] We hypothesized that if the supplement was effective at inducing a weight loss, malondialdehyde would be lowered. To the contrary, malondialdehyde was increased slightly in the supplement condition from pre to post intervention despite the loss in body weight and body fat, and more so in the

Table 4. Metabolic panel data for 32 men and women assigned to OxyELITE Pro™ or placebo for eight weeks.

Variable	OxyELITE Pro™		Placebo	
	Pre (n = 16)	Post (n = 16)	Pre (n = 16)	Post (n = 16)
Glucose ($mg \cdot dL^{-1}$)*	87.8 ± 1.2	88.3 ± 1.6	85.4 ± 1.2	83.8 ± 2.1
BUN ($mg \cdot dL^{-1}$)	13.8 ± 1.0	14.6 ± 1.1	14.0 ± 1.2	15.3 ± 0.9
Creatinine ($mg \cdot dL^{-1}$)	1.0 ± 0.0	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.0
BUN:creatinine	14.0 ± 1.0	14.4 ± 1.1	13.8 ± 1.0	15.8 ± 0.8
Sodium ($mmol \cdot L^{-1}$)	140.2 ± 0.6	139.9 ± 0.4	139.5 ± 0.7	140.1 ± 0.4
Potassium ($mmol \cdot L^{-1}$)*	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.7 ± 0.1
Chloride ($mmol \cdot L^{-1}$)	103.4 ± 0.5	103.0 ± 0.5	103.1 ± 0.7	102.6 ± 0.5
CO_2 ($mmol \cdot L^{-1}$)†	24.6 ± 0.5	27.4 ± 0.5	24.6 ± 0.7	26.1 ± 0.4
Calcium ($mg \cdot dL^{-1}$)	9.4 ± 0.1	9.5 ± 0.1	9.3 ± 0.1	9.3 ± 0.1
Protein ($g \cdot dL^{-1}$)	6.9 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	6.9 ± 0.1
Albumin ($g \cdot dL^{-1}$)	4.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.1
Globulin ($g \cdot dL^{-1}$)	2.5 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.5 ± 0.1
A:G	1.8 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.0
Bilirubin ($mg \cdot dL^{-1}$)	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Alk Phos ($IU \cdot L^{-1}$)*	77.6 ± 5.8	73.8 ± 6.0	62.1 ± 5.4	60.8 ± 4.4
AST (SGOT) ($IU \cdot L^{-1}$)	24.3 ± 2.3	22.4 ± 1.9	23.9 ± 2.2	23.1 ± 2.6
ALT (SGPT) ($IU \cdot L^{-1}$)	20.9 ± 2.1	20.5 ± 2.2	19.6 ± 2.1	19.0 ± 1.5
GGT ($IU \cdot L^{-1}$)	17.9 ± 3.6	17.0 ± 2.0	13.4 ± 1.2	14.8 ± 1.0

Notes: Values are mean ± SEM. No condition × Pre/Post intervention interaction effects noted ($P > 0.05$). *Condition effect noted for glucose ($P = 0.02$), potassium ($P = 0.05$), and alkaline phosphatase ($P = 0.01$); trend noted for calcium ($P = 0.08$). No other condition effects noted ($P > 0.05$); †Pre/Post intervention effect noted for CO_2 ($P = 0.0002$). No other Pre/Post intervention effects noted ($P > 0.05$).

Table 5. Appetite and dietary intake for 32 men and women assigned to OxyELITE Pro™ or placebo before and during the final week of an eight week intervention.

Variable	OxyELITE Pro™		Placebo	
	Pre (n = 16)	Post (n = 16)	Pre (n = 16)	Post (n = 16)
Appetite (1–10 scale)†	6.3 ± 0.3	4.8 ± 0.5	6.0 ± 0.2	5.7 ± 0.2
Kilocalories	2359.0 ± 284.2	1930.0 ± 203.7	2419.5 ± 218.4	1964.8 ± 202.7
Protein (g)	99.6 ± 13.3	89.5 ± 9.7	106.5 ± 13.1	90.6 ± 10.3
Carbohydrate (g)	276.6 ± 28.9	229.1 ± 20.9	284.8 ± 32.7	241.2 ± 26.7
Fiber (g)	16.4 ± 1.4	16.5 ± 2.7	20.4 ± 3.0	15.1 ± 1.7
Sugar (g)	101.7 ± 14.1	77.9 ± 8.7	114.7 ± 19.8	99.1 ± 17.3
Fat (g)	70.1 ± 9.3	70.9 ± 10.6	89.2 ± 9.6	67.2 ± 8.4
Saturated fat (g)	22.4 ± 2.8	21.7 ± 2.8	28.0 ± 3.3	22.1 ± 3.0
Monounsaturated fat (g)	10.8 ± 1.7	12.3 ± 2.1	16.6 ± 3.7	13.2 ± 2.5
Polyunsaturated fat (g)	5.0 ± 0.8	6.1 ± 0.9	8.2 ± 1.6	5.4 ± 0.9
Trans fat (g)	0.9 ± 0.2	1.4 ± 0.5	1.5 ± 0.4	1.4 ± 0.4
Cholesterol (mg)	323.3 ± 54.3	365.9 ± 69.9	315.0 ± 62.0	306.5 ± 64.8
Vitamin C (mg)	63.9 ± 9.8	53.0 ± 11.0	79.2 ± 14.1	51.4 ± 9.0
Vitamin E (mg)	4.5 ± 2.2	5.1 ± 1.7	5.0 ± 1.8	3.8 ± 1.2
Vitamin A (RE)	369.7 ± 98.0	335.3 ± 77.5	265.5 ± 50.9	345.0 ± 73.2
Selenium (μg)	35.8 ± 6.4	52.1 ± 9.8	47.4 ± 7.9	59.7 ± 10.4

Notes: Values are mean ± SEM. No significant condition × Pre/Post intervention interaction effects noted ($P > 0.05$); trend noted for appetite ($P = 0.08$). No significant condition effects noted ($P > 0.05$); †Pre/Post intervention effect noted for appetite ($P = 0.01$); appetite lower from pre to post intervention for OxyELITE Pro™ (paired *t*-test; $P = 0.0006$); trend noted for kilocalories ($P = 0.06$); total kilocalories lower from pre to post intervention for placebo (paired *t*-test; $P = 0.004$); No other significant pre/post intervention effects noted ($P > 0.05$).

placebo condition. This may be at least partly due to the lowering of kilocalories from pre to post intervention (Table 5), a suggestion supported by recent work indicating an increase in malondialdehyde following caloric restriction in mice.[18] Aside from this, potential changes in subjects' activity profile during the days leading up the test days, coupled with the possibility of variation in sample processing and analysis may have contributed to the noted differences.

Second, total and HDL-C was increased in the supplement condition from pre to post intervention. Despite the increase in total cholesterol, the LDL-C:HDL-C and total cholesterol:HDL-C were lowered. Considering the benefits of HDL-C,[19] an improvement in this measure, and in particular the measures of LDL-C:HDL-C and total cholesterol:HDL-C, is welcome. This finding, coupled with the reduction in adiposity may suggest a cardioprotective effect of the supplement. It should be noted that a significant increase was also observed in HDL-C for the placebo condition, while a decrease was noted in LDL-C:HDL-C and total cholesterol:HDL-C. It is possible that these findings were due to changes in dietary intake over the course of the study (eg, reduction in kilocalorie intake). While no statistically significant interactions or main effects were noted for any dietary variables, total kilocalories were lower for placebo when comparing pre and post intervention data. A similar trend for lower kilocalorie intake was observed for the supplement condition, highlighting the possibility that overall dietary intake may have impacted the findings for serum lipids.

It should be understood that although subjects completed seven day food logs during the week before starting the intervention and during the final week of the intervention, they did not record all food and beverage consumed during the entire study period. While subjects were instructed to maintain their usual diet throughout the study period, it is possible that dietary intake varied over the course of the eight week intervention. Hence, either of the two weeks of collection may have underestimated or overestimated subjects' usual intake during their entire participation. This lack of control of dietary intake is indeed a limitation of our work, and of all similar human subject research.

The supplement tested in the present investigation contains six active ingredients (caffeine, bauhinia purpurea, bacopa monniera, geranium stem extract [1,3 dimethlyamlamine], cirsium oligophyllum, and rauwolscine extract), for which a detailed explanation of their potential mechanisms of action has been previously provided.[12] We cannot state with confidence which of these ingredients is chiefly responsible for the results obtained herein. We can simply state that the combination of these ingredients yields the results presented; which applies for all variables including anthropometric, bloodborne, and appetite.

One unique aspect of our study was our selected subject sample. That is, unlike most investigations using a dietary ingredient or whole food intervention in an attempt to induce weight loss, which typically include obese subjects exclusively (often who are sedentary), the present investigation used active individuals of whom 15 were normal weight (BMI 18.5–24.9 $kg \cdot m^{-2}$), 13 were overweight (BMI 25–29.9 $kg \cdot m^{-2}$), and 4 were obese (BMI $\geq$ 30 $kg \cdot m^{-2}$). Although we likely would have observed more robust changes in our outcome measures if we included obese subjects exclusively, we believe that our approach has much more application, in that our findings may have relevance to a large majority of individuals currently exercising with the desire to lose additional body weight and body fat.

With regards to our collected safety data, we noted an increase in resting heart rate of six beats per minute without a significant accompanying increase in systolic or diastolic blood pressure (although values for both blood pressure measures increased ~3 mmHg from pre to post intervention). Interestingly, we have noted a slight *decrease* in resting heart rate and blood pressure in a prior study of OxyELITE Pro™, when subjects ingested the supplement each day for two weeks.[13] Based on these conflicting findings, we are uncertain as to what the typical change in hemodynamic variables is following use of this supplement. The data for chronic caffeine intake (often delivered within coffee—at an amount equal to ~200–400 mg, or the equivalent of 2–4 cups) in relation to hemodynamic variables are mixed,[20] with some reports indicating a slight increase in these measures and others indicating no change or a slight decrease. Considering that other components are included within the tested dietary supplement besides caffeine, additional study would be necessary to either confirm or refute our initial work pertaining to the chronic effects of the supplement on hemodynamic variables. The small

increase in heart rate and blood pressure noted in the present study may be considered acceptable by some individuals, in particular those who are healthy with a low cardiovascular disease risk profile.

Regarding other bloodborne variables, we noted minimal change in all measured variables (Tables 3 and 4), with no interaction effects noted except for monocytes, which were higher post intervention for the supplement condition. Of particular importance concerning oral dietary supplements is the measure of liver enzymes. There was no increase noted in SGOT, SGPT, or GGT from pre to post intervention for the supplement condition. In fact, all values decreased slightly. Collectively, these findings indicate that eight weeks of intake of the dietary supplement at a dosage of one to two capsules per day does not cause any adverse outcomes in a sample of young and healthy men and women, with the exception of an increase in resting heart rate.

Conclusion

In conclusion, our findings indicate that the dietary supplement OxyELITE Pro™ may aid in weight and body fat loss in young, exercise-trained men and women. While the supplement does not adversely affect bloodborne markers of safety or increase resting blood pressure significantly, it does elevate resting heart rate. As the majority of subjects in this investigation were not obese, it is possible that supplementation with this agent could provide more robust effects in those with higher body weight and fat mass. Additional investigation is needed to confirm this hypothesis.

Competing Interests

RJB has received research funding or acted as consultant to nutraceutical and dietary supplement companies. Other authors declare no competing interests.

Author Contributions

CGM and REC coordinated the study and were responsible for data collection. RJA performed anthropometric measures and assisted with data collection. JPR performed DEXA scans. RJB was responsible for the study design, overseeing data collection, biochemical work, statistical analysis, and preparation of the manuscript. All authors reviewed and approved of the final manuscript.

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Disclosures

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ORIGINAL RESEARCH

Impact of a Dietary Supplement Containing 1,3-Dimethylamylamine on Blood Pressure and Bloodborne Markers of Health: a 10-Week Intervention Study

Paul N. Whitehead, Brian K. Schilling, Tyler M. Farney and Richard J. Bloomer

Cardiorespiratory/Metabolic Laboratory, The University of Memphis, Memphis, TN 38152, USA.
Corresponding author email: rbloomer@memphis.edu

Abstract

Background: 1,3-dimethylamylamine is a commonly used ingredient within dietary supplements. Our prior work with this agent indicates a transient increase in blood pressure (systolic in particular) following oral ingestion of a single dosage, but no significant increase in resting blood pressure following chronic ingestion. Moreover, intervention studies involving both two and eight weeks of treatment with finished products containing 1,3-dimethylamylamine indicate minimal or no change in bloodborne markers of health. The present study sought to extend these findings by using a 10-week intervention trial to determine the change in selected markers of health in a sample of men.

Methods: 25 healthy men were randomly assigned to either a placebo (n = 13) or to a supplement containing 1,3-dimethylamylamine (n = 12) for a period of 10 weeks. Before and after the intervention, resting blood pressure and heart rate were measured, and blood samples were collected for determination of complete blood count, metabolic panel, and lipid panel.

Results: No significant differences were noted between conditions for blood pressure ($P > 0.05$), although systolic blood pressure increased approximately 6 mmHg with the supplement (diastolic blood pressure decreased approximately 4 mmHg). A main effect for time was noted for heart rate ($P = 0.016$), with values decreasing from pre to post intervention. There were significant main effects for time for creatinine (increased from pre to post intervention; $P = 0.043$) and alkaline phosphatase (decreased from pre to post intervention; $P = 0.009$), with no condition differences noted ($P > 0.05$). There was a significant interaction noted for low density lipoprotein cholesterol (LDL-C) ($P = 0.043$), with values decreasing in the supplement group from pre to post intervention approximately 7 $mg \cdot dL^{-1}$ ($P = 0.034$). No other effects of significance were noted for bloodborne variables.

Conclusion: These data indicate that a dietary supplement containing 1,3-dimethylamylamine does not result in a statistically significant increase in resting heart rate or blood pressure (although systolic blood pressure is increased ~6 mmHg with supplement use). The supplement does not negatively impact bloodborne markers of health. Further study is needed involving a longer intervention period, a larger sample size, and additional measures of health and safety.

Keywords: nutritional supplements, chronic effects, 1,3-dimethylamylamine

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Background

While somewhat controversial, 1,3-dimethylamylamine has been suggested to be a component of the Pelargonium graveolens plant;[1] a simple aliphatic amine[2] with sympathomimetic properties. Aside from 1,3-dimethylamylamine, common chemical names cited for this agent include 2-amino-4-methylhexane, 1,3-dimethylpentylamine, methylhexaneamine, and 4-methyl-2-hexylamine. It is also known by the trademarked name geranamine™ (Proviant Technologies, Inc, 2005: US trademark number: 78542697).

The use of 1,3-dimethylamylamine is widespread within the dietary supplement industry, in particular as a component of weight loss and pre-workout products. A recent report published by the Human Performance Resource Center (http://www.humanperformanceresourcecenter.org) provides a detailed listing of close to 100 products believed to contain 1,3-dimethylamylamine. While anecdotal reports of improved exercise performance are common, we are aware of only one published experiment designed to investigate the ergogenic properties of 1,3-dimethylamylamine alone or in combination with caffeine,[3] which noted little impact on aerobic exercise performance. Regardless of actual ergogenic effectiveness, the overall safety of the ingredient needs to be considered.

In our recent article in which 1,3-dimethylamylamine was administered alone and in combination with caffeine in a single dosage to healthy men and women,[4] we noted that heart rate was unaffected by treatment, but blood pressure was elevated when consuming 1,3-dimethylamylamine—generally in a dose-dependent manner. The peak percent change in systolic (~20%) and diastolic (~17%) blood pressure was noted at 60 minutes following ingestion of the combination of 250 mg of caffeine and 75 mg of 1,3-dimethylamylamine. A second study investigated the blood pressure and heart rate response of 1,3-dimethylamylamine and caffeine alone and in combination (compared to a placebo) in a sample of endurance trained men and women before, during, and following strenuous exercise.[3] In this study, heart rate was relatively similar across conditions, and blood pressure was generally highest for caffeine and 1,3-dimethylamylamine alone compared to other conditions.

Research on the use of 1,3-dimethylamylamine in combination with other ingredients within finished products demonstrated first that, in terms of acute changes in blood pressure and heart rate, dietary supplements containing 1,3-dimethylamylamine result in minimal change in heart rate but do increase systolic blood pressure transiently.[5,6] Second, in terms of *chronic* changes in blood pressure and heart rate, we have noted little or no change in resting measures after a two-week[5] or eight-week[7] intervention. Third, in terms of chronic changes in bloodborne biomarkers of health and safety, we have noted little or no change in measures after a two-week[5] or eight-week[7] intervention.

Collectively, the above findings indicate relative safety of this ingredient, at least with regard to the included outcome measures. However, due to the transient increase in systolic blood pressure, the need exists to further evaluate the potential for this agent to raise resting blood pressure following a longer period of chronic intake. The present study sought to extend our prior findings related to the use of 1,3-dimethylamylamine in a combined product by using a 10-week intervention trial to determine the change in selected markers of health and safety in a sample of healthy men.

Methods

Subjects

Thirty resistance-trained men were recruited from the local university campus and surrounding community. Sample size was chosen based on studies of similar magnitude and scope; however a power analysis was not performed *a priori*. Subjects were self-reported non-smokers, and did not have cardiovascular or metabolic disease. Health history, drug and dietary supplement usage, and physical activity questionnaires were completed by subjects. Subjects were instructed to maintain their current exercise training and dietary intake programs throughout the study period with the exception of refraining from strenuous exercise during the 48 hours prior to each lab session. Subjects did not use dietary supplements, with the exception of meal replacements and protein powders, during the study period. The study was approved by the University of Memphis Committee for Human Subject Research.

Design

This study involved a randomized, placebo-controlled design. Although the study was also double-blinded, the stimulant effects of the supplement suggest that

blinding from the subject standpoint is essentially lost. During the first visit to the laboratory, subjects provided written informed consent and completed health and physical activity questionnaires.

Lab protocol

Subjects reported to the lab in the morning hours following a minimum of an eight-hour overnight fast, before and after the 10-week intervention. Upon arrival to the lab, subjects rested quietly in a supine position for 10 minutes. Heart rate (via palpation) and blood pressure (via stethoscope) were then measured and recorded. A blood sample was then obtained from subjects (~20 mL). Blood was processed and sent to Laboratory Corporation of America for analysis of complete blood count, comprehensive metabolic panel, and lipid panel. The complete blood count was determined using an automated cell counter (Coulter LH750). The comprehensive metabolic panel was determined using automated procedures (Roche/Hitachi Modular). The lipid panel was determined using enzymatic procedures (Roche/Hitachi Modular). To better characterize subjects, body mass (using an electronic scale) and body composition (using dual energy x-ray absorptiometry) was measured. These same procedures were used both pre and post intervention for all subjects.

Physical activity and dietary intake

Subjects were asked to maintain their usual physical activity and dietary patterns and to record all food and drink consumed during the seven days prior to each lab session (both pre and post intervention). All records were analyzed for total calories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A (Food Processor SQL, version 9.9, ESHA Research, Salem, OR).

Conditions

The two conditions consisted of a placebo powder (microcrystalline cellulose, silicon dioxide, citric acid, natural flavor, acesulfame-K, sucralose, vegetable stearate, chlorophyll for coloring; at a volume equal to that of the supplement) and a dietary supplement containing a proprietary blend of 1,3-dimethylamylamine, caffeine, creatine monohydrate, β-alanine, schinzandrol A, and arginine alpha-ketoglutarate (Jack3d™, USPlabs, LLC. Dallas, TX). Subjects were instructed to consume 1–3 servings on each *workout day*, 30 minutes prior to their exercise session. The mean number of workout days per week for subjects was four and the supplement was not consumed on non-exercise days. Subjects reported back to the laboratory as needed for receipt of an additional container of their assigned condition. Both the supplement and placebo powder were to be mixed into eight ounces of water and both had a similar lemon-lime taste. Subjects ingested the assigned condition for the duration of the 10-week study period.

Statistical analysis

Data were analyzed using a 2 (condition) × 2 (time) factorial analysis of variance (ANOVA) with use of Fisher's LSD. Data are presented as mean ± standard deviation. All analyses were performed using PASW statistical software (version 18). Statistical significance was set at $P \leq 0.05$. Standardized effect sizes (ES) were calculated for means comparisons.[8] Effect size magnitudes can be interpreted as follows: 0.2 small, 0.6 moderate, and 1.2 large.

Results

Although 30 subjects began the study, only 25 subjects successfully completed all lab sessions. Of the five subjects who failed to complete the study, two were initially assigned to the placebo and three were initially assigned to the supplement. One subject in the supplement group was injured while training and was unable to complete the intervention, and thus was excluded from analysis. The remaining four subjects failed to complete all testing due to personal reasons. These five subjects are not included in the analyses. Additionally, two subjects in the placebo group were unable to provide blood samples pre- and post intervention and are thus excluded from the bloodborne variable analysis. No subjects reported adverse events attributable to the supplement or placebo.

Of the 25 subjects in the analysis, self reported use data of subjects indicate that the mean number of scoops of the supplement consumed on training days was 2.4 ± 0.3 (2 subjects = 3 scoops; 7 subjects = 2 scoops; 3 subjects = 1.5 scoops), which was greater than ($P = 0.0003$) the mean number of scoops of the placebo consumed (1.7 ± 0.4; 7 subjects = 2 scoops; 3 subjects = 1.5 scoops; 3 subjects = 1 scoops). Subject characteristics are presented in Table 1.

Table 1. Characteristics of men assigned to supplement (N = 12) or placebo (N = 13) for ten weeks.

Variable	Supplement		Placebo	
	Pre	Post	Pre	Post
Age (years)	23 ± 2.9	NA	21.9 ± 2.3	NA
Weekly resistance training (hrs)	5.2 ± 1.9	NA	4.4 ± 1.7	NA
Resistance training history (yrs)	3.6 ± 3.5	NA	4.2 ± 1.7	NA
Body weight (kg)	76.9 ± 9.1	77.2 ± 10.1	80.7 ± 15.6	82.0 ± 16.1
DEXA total body fat (%)	17.0 ± 4.9	15.8 ± 5.2	15.6 ± 9.2	16.2 ± 9.7
Heart rate (bpm)*	61.5 ± 12.9	58.4 ± 6.4	61.4 ± 10.6	55.2 ± 8.3
Systolic blood pressure (mmHg)	117.8 ± 10.6	124.4 ± 19.3	121.2 ± 12.3	119.6 ± 8.6
Diastolic blood pressure (mmHg)	76.3 ± 9.2	72.0 ± 7.9	76.0 ± 8.4	75.3 ± 5.8

Notes: Values are mean ± SD. *Significant main effect for time ($P < 0.05$).

Blood pressure and heart rate data

No significant differences were noted between conditions for blood pressure ($P > 0.05$). However, systolic blood pressure increased approximately 6 mmHg with the supplement, while diastolic blood pressure decreased approximately 4 mmHg from pre to post intervention. A main effect for time was noted for heart rate ($P = 0.016$, ES = 1.1), with values decreasing from pre to post intervention. No other findings were of statistical significance ($P > 0.05$). Blood pressure and heart rate data are presented in Table 1.

Bloodborne data

There were significant main effects for time for creatinine (increased from pre to post intervention; $P = 0.043$, ES = 1.1) and alkaline phosphatase (decreased from pre to post intervention; $P = 0.009$, ES = 0.5), with no condition differences noted ($P > 0.05$). There was a significant interaction noted for low density lipoprotein cholesterol (LDL-C) ($P = 0.043$), with values decreasing in the supplement group from pre to post intervention approximately 7 $mg \cdot dL^{-1}$ ($P = 0.034$, ES = 0.9). No other effects of statistical significance were noted for bloodborne variables. Data are presented in Table 2 for complete blood count, Table 3 for metabolic panel, and Table 4 for lipid panel.

Dietary data

There was a significant main effect for time for dietary fiber ($P = 0.018$, ES = 0.8), which decreased from pre to post intervention. A main effect for time was also noted for dietary selenium ($P = 0.032$, ES = 0.8), which increased from pre to post intervention. Dietary data are presented in Table 5.

Table 2. Complete blood count data for men assigned to supplement (N = 12) or placebo (N = 11) for ten weeks.

Variable	Supplement		Placebo	
	Pre	Post	Pre	Post
WBC ($10^3 \cdot \mu L^{-1}$)	5.8 ± 1.5	5.2 ± 1.0	5.2 ± 1.7	4.8 ± 1.1
RBC ($10^6 \cdot \mu L^{-1}$)	5.1 ± 0.4	4.9 ± 0.3	4.8 ± 0.3	4.8 ± 0.3
Hemoglobin ($g \cdot dL^{-1}$)	15.3 ± 1.0	14.8 ± 0.9	14.6 ± 0.7	14.6 ± 0.8
Hematocrit (%)	45.4 ± 2.6	44.2 ± 2.0	43.8 ± 1.6	43.3 ± 2.0
MCV (fL)	89.8 ± 4.9	89.7 ± 4.7	91.2 ± 3.3	90.2 ± 4.0
MCH (pg)	30.3 ± 2.2	30.1 ± 2.2	30.4 ± 1.7	30.4 ± 1.3
MCHC ($g \cdot dL^{-1}$)	33.7 ± 0.9	33.6 ± 0.8	33.4 ± 1.1	33.6 ± 0.6
RDW (%)	13.0 ± 0.6	12.9 ± 0.4	13.1 ± 0.4	12.9 ± 0.6
Platelets ($10^3 \cdot \mu L^{-1}$)	244.5 ± 32.5	234.9 ± 35.3	200.6 ± 42.3	194.6 ± 33.1
Neutrophils (%)	49.8 ± 9.3	47.6 ± 6.6	50.5 ± 11.8	46.1 ± 11.2
Lymphocytes (%)	37.1 ± 8.1	37.6 ± 6.9	35.9 ± 11.3	39.7 ± 11.6
Monocytes (%)	9.2 ± 1.7	9.8 ± 2.1	10.2 ± 2.7	10.4 ± 1.4
Eosinophils (%)	3.4 ± 2.5	4.3 ± 3.4	2.8 ± 1.8	3.2 ± 1.7
Basophils (%)	0.5 ± 0.5	0.7 ± 0.5	0.6 ± 0.7	0.6 ± 0.7

Notes: Values are mean ± SD. No differences of statistical significance noted ($P > 0.05$).

Table 3. Comprehensive metabolic panel data for men assigned to supplement (N = 12) or placebo (N = 11) for ten weeks.

Variable	Supplement		Placebo	
	Pre	Post	Pre	Post
Glucose ($mg \cdot dL^{-1}$)	92.3 ± 5.9	90.1 ± 8.9	83.8 ± 7.5	88.5 ± 6.3
BUN ($mg \cdot dL^{-1}$)	17.0 ± 5.3	17.0 ± 4.7	15.6 ± 2.5	18.0 ± 3.4
Creatinine ($mg \cdot dL^{-1}$)*	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
BUN:creatinine	15.3 ± 3.8	14.8 ± 4.1	14.7 ± 3.1	16.0 ± 2.6
Sodium ($mmol \cdot L^{-1}$)	141.3 ± 1.7	141.3 ± 1.9	142.0 ± 1.3	140.9 ± 1.8
Potassium ($mmol \cdot L^{-1}$)	4.7 ± 0.5	4.5 ± 0.3	4.4 ± 0.4	4.3 ± 0.5
Chloride ($mmol \cdot L^{-1}$)	102.9 ± 1.3	103.7 ± 1.5	103.3 ± 2.1	103.4 ± 1.5
CO_2 ($mmol \cdot L^{-1}$)	26.4 ± 1.6	26.9 ± 2.1	26.7 ± 1.9	26.6 ± 1.7
Calcium ($mg \cdot dL^{-1}$)	9.5 ± 0.2	9.3 ± 0.2	9.3 ± 0.3	9.3 ± 0.3
Protein ($g \cdot dL^{-1}$)	6.7 ± 0.3	6.7 ± 0.3	6.6 ± 0.3	6.7 ± 0.3
Albumin ($g \cdot dL^{-1}$)	4.5 ± 0.1	4.5 ± 0.2	4.5 ± 0.2	4.5 ± 0.3
Globulin ($g \cdot dL^{-1}$)	2.2 ± 0.3	2.2 ± 0.3	2.1 ± 0.2	2.2 ± 0.2
A:G	2.0 ± 0.4	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.3
Bilirubin ($mg \cdot dL^{-1}$)	0.7 ± 0.4	0.6 ± 0.3	1.1 ± 0.8	1.0 ± 0.9
Alk Phos ($IU \cdot L^{-1}$)*	74.8 ± 22.8	70.5 ± 20.0	63.6 ± 17.1	59.9 ± 14.6
AST (SGOT) ($IU \cdot L^{-1}$)	23.9 ± 5.0	23.3 ± 3.9	23.3 ± 7.0	22.3 ± 5.9
ALT (SGPT) ($IU \cdot L^{-1}$)	23.5 ± 11.7	23.8 ± 7.7	22.6 ± 13.2	20.3 ± 10.2
GGT ($IU \cdot L^{-1}$)	19.6 ± 8.1	17.8 ± 7.0	18.9 ± 12.7	18.9 ± 13.1

Notes: Values are mean ± SD. *Significant main effect for time ($P < 0.05$).

Discussion

Our data indicate that a dietary supplement containing 1,3-dimethylamylamine does not significantly increase resting heart rate or blood pressure (although systolic blood pressure increased ~6 mmHg with the supplement). Moreover, the supplement does not adversely impact bloodborne biomarkers of health, but rather, results in a decrease in LDL-C. These findings are in reference to a small sample of healthy men who exercise regularly. Due to the fact that our sample size is small, additional well-designed experiments of similar scope, inclusive of larger sample sizes, are needed to extend the findings presented within. It is only through such work that our ability to generalize these findings to the population at large will be possible.

These data extend our prior work using 1,3-dimethylamylamine in combination with other ingredients, including those considered to be stimulants.[5,7] Collectively, these data provide some support for the relative safety of this agent; at least with regards to the measured outcomes used in this study, as well as others which we have conducted. Despite this, more work is needed involving a longer intervention period and the inclusion of additional measures of health (eg, toxicology, cardiac function), to more fully elucidate the safety of oral 1,3-dimethylamylamine ingestion. Additionally, since products containing

Table 4. Lipid panel data for men assigned to supplement (N = 12) or placebo (N = 11) for ten weeks.

Variable	Supplement		Placebo	
	Pre	Post	Pre	Post
Cholesterol ($mg \cdot dL^{-1}$)	163.2 ± 25.7	154.5 ± 20.6	148.5 ± 20.4	149.3 ± 21.0
Triglycerides ($mg \cdot dL^{-1}$)	88.6 ± 36.3	83.6 ± 28.3	65.2 ± 26.7	68.8 ± 19.0
HDL-C ($mg \cdot dL^{-1}$)	53.1 ± 15.6	52.5 ± 12.9	53.4 ± 16.5	50.7 ± 10.4
VLDL-C ($mg \cdot dL^{-1}$)	17.8 ± 7.4	16.6 ± 5.7	13.1 ± 5.4	13.8 ± 3.8
LDL-C ($mg \cdot dL^{-1}$)*	92.3 ± 21.6	85.2 ± 17.5	82.0 ± 14.6	84.7 ± 16.9
LDL-C/HDL-C	1.9 ± 0.7	1.7 ± 0.5	1.7 ± 0.6	1.7 ± 0.4

Notes: Values are mean ± SD. *Significant interaction ($P < 0.05$); lower for supplement from pre to post intervention ($P = 0.034$).

Table 5. Dietary intake for men assigned to supplement (N = 12) or placebo (N = 13) for ten weeks.

Variable	Supplement		Placebo	
	Pre	Post	Pre	Post
Kilocalories	2721.3 ± 468.0	2619.0 ± 705.0	2405.9 ± 561.4	2262.4 ± 457.7
Protein (g)	135.2 ± 44.5	126.5 ± 41.5	101.3 ± 27.9	110.4 ± 27.9
Carbohydrate (g)	299.0 ± 93.7	295.4 ± 131.4	310.5 ± 95.2	264.1 ± 68.1
Fiber (g)*	23.9 ± 9.8	21.1 ± 12.4	21.0 ± 7.9	17.2 ± 4.7
Sugar (g)	92.4 ± 46.0	103.9 ± 62.3	97.8 ± 48.0	83.1 ± 34.3
Fat (g)	104.1 ± 18.3	101.1 ± 20.1	82.0 ± 27.3	81.8 ± 18.4
Saturated fat (g)	30.6 ± 8.2	29.4 ± 5.9	25.1 ± 11.9	24.5 ± 6.9
Monounsaturated fat (g)	22.8 ± 10.8	22.8 ± 14.2	13.6 ± 5.8	15.9 ± 4.9
Polyunsaturated fat (g)	9.7 ± 5.5	8.8 ± 4.0	6.4 ± 5.8	7.4 ± 2.8
Cholesterol (mg)	412.1 ± 254.8	427.5 ± 245.0	286.1 ± 110.8	340.1 ± 178.4
Vitamin C (mg)	148.6 ± 149.6	114.6 ± 157.9	133.3 ± 99.2	109.5 ± 59.6
Vitamin E (mg)	8.1 ± 8.2	8.5 ± 8.8	5.7 ± 5.3	6.0 ± 4.6
Vitamin A (RE)	382.0 ± 285.8	369.9 ± 379.2	303.3 ± 179.3	348.7 ± 235.3
Selenium (μg)*	47.6 ± 24.0	55.3 ± 34.9	42.2 ± 33.3	59.9 ± 41.8

Notes: Values are mean ± SD. *Significant main effect for time ($P < 0.05$).

1,3-dimethylamylamine are marketing not only to men but also to women, more work using a larger sample of women may be considered.

The majority of subjects in the present study consumed the supplement at a dosage of two scoops per training day. No adverse events were noted and the supplement was well-tolerated based on subject self-report. This agrees with our prior work with this same supplement, in which all subjects successfully completed a two-week intervention period, without incident, in which two-scoops of the supplement were consumed daily.[5]

As can be viewed in Table 1, systolic blood pressure increased approximately 6 mmHg with the supplement, while diastolic blood pressure decreased approximately 4 mmHg from pre to post intervention. Neither change was of statistical significance. Heart rate was lower from pre to post intervention, which would subsequently impact the rate pressure product (systolic blood pressure × heart rate). That is, when calculating the rate pressure product values at pre (7245) and post (7265) intervention (using the mean data provided in Table 1), it is noted that values are near identical. This may be of interest to those with concern over the increase in systolic blood pressure and the potential for increased myocardial stress.

Findings of minimal change in heart rate and blood pressure (and hence, rate pressure product) have been noted in our two-week intervention using the same supplement.[5] Little change in blood pressure was noted in our eight week intervention using another supplement containing 1,3-dimethylamylamine,[7] while an increase of ~6 bpm in resting heart rate have been noted. Interestingly, in our two-week intervention using another supplement containing 1,3-dimethylamylamine,[5] resting heart rate was decreased by ~4 bpm. More work is needed to better understand the role of 1,3-dimethylamylamine in relation to hemodynamic variables.

Related to bloodborne variables, we included the routine panels as used as part of a physical examination (ie, complete blood count, metabolic panel, lipid panel). No adverse effects were noted for any measured variable, including those related to liver function (eg, SGOT, SGPT, GGT). Variables were very similar from pre to post intervention for the supplement group. Creatinine was noted to be higher from pre to post intervention, in particular for the supplement group (Table 3). This small increase may have been due to the inclusion of creatine within the dietary supplement. The measures of triglycerides, total and LDL cholesterol decreased slightly from pre to post intervention, with statistically significant findings noted only for LDL-C (Table 4). As with blood pressure and heart rate, these bloodborne data extend our prior work involving a two-week intervention study using the same supplement[5] (with the exception of an increase in fasting blood glucose noted in our prior work), as well as in our eight week intervention study using a different supplement containing 1,3-dimethylamylamine.[7]

Conclusion

Findings from the present study indicate that a dietary supplement containing 1,3-dimethylamylamine consumed for a period of 10 weeks does not result in a statistically significant increase in resting heart rate or blood pressure in a sample of healthy men, nor does the supplement negatively impact bloodborne biomarkers of health. The supplement results in a small decrease in LDL-C, which may suggest a potential cardioprotective role. While this may have clinical relevance, additional studies inclusive of larger samples are needed to replicate these findings, as well as to extend and expand upon other findings associated with this work. In particular, longer intervention periods and the inclusion of additional measures of health and toxicity are needed in future studies involving 1,3-dimethylamylamine. Finally, it should be noted that despite our lack of statistical significance, the supplement did result in a mean increase in systolic blood pressure of ~6 mmHg, indicating that it would be prudent for those with elevated blood pressure to avoid use of dietary supplements containing 1,3-dimethylamylamine.

Competing Interests

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

Author Contributions

PNW, BKS, and TMF were responsible for data collection/entry/analysis and assistance with manuscript preparation. RJB was responsible for the study design and preparation of the manuscript. All authors read and approved the final manuscript.

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THE COMPARATIVE PHARMACOLOGY OF THE ISOMERIC NITROGEN METHYL SUBSTITUTED HEPTYLAMINES

DAVID F. MARSH, A. HOWARD AND D. A. HERRING

Department of Pharmacology, West Virginia University School of Medicine, Morgantown

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Since the fundamental study of Barger and Dale (1910) of the relationship between the chemical constitution and physiological action of the sympathomimetic amines, numerous investigations have been made of many structurally and pharmacologically related compounds. Several of these investigations have been concerned with the influence on activity brought about by the substitution of a methyl group for one of the hydrogen atoms on the amino group of various beta-phenylalkylamines (Beyer, 1946) and a few with this change in cycloaliphatic amines (Swanson and Chen, 1948; Marsh, 1948a). No systematic study of this structural change in the field of simple aliphatic amines has been made, although one N-methyl aliphatic amine, 2-heptyl methylamine[1] or Oenethyl, is generally available.

In this study, the various nitrogen methyl heptylamines have been compared with the unsubstituted amines (see table 1)[2]. By limiting the problem to compounds with a total of six plus one carbon atoms in the primary structural unit, it is possible to have a wide range of activity and to determine any relationship between general spatial configuration and the influence of N-methylation without having to consider differences in molecular weight. N-methylation increases the molecular weight 12 per cent in this group of agents and this is probably less than the error of the most accurate procedure used in the investigation.

EXPERIMENTAL. Since repeated injections of the heptylamines yield diminished effects in animals (Marsh, 1948b), the following cross-over, indirect, method was used to avoid any possible error that could be introduced by such tachyphylaxis.

Nine mongrel dogs weighing 7.1–16 kgm. were given 20 mgm. of sodium thiopental per kgm. intravenously. A femoral artery and vein were quickly exposed; a hypodermic needle cannula attached to a Statham strain gage manometer and General Electric recording microammeter (Marsh, 1949) was introduced into the artery; one mgm. of scopolamine hydrobromide per kgm. was given; this was followed by 2, 4, 5, or 7 microgm. epinephrine

[1] This material is frequently referred to as "2-methylamino heptane." Since the naming of an organic compound possessing one primary functional group as a derivative of its parent hydrocarbon violates the fundamental nomenclature rules of the Commission on the Reform of the Nomenclature of Organic Chemistry of the International Union of Chemistry (cf. J. Am. Chem. Soc., **55**: 3905–25, 1933), we have referred to this compound by the name 2-heptyl methylamine.

[2] We are grateful to Dr. K. K. Chen and Mr. H. A. Shonle of the Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, Indiana for the sulfate salts of the unsubstituted amines (except 2-methyl-2-hexylamine). These were converted to the free base and dissolved in the theoretical amount of hydrochloric acid to make ten per cent solutions. We are grateful to Dr. R. O. Hauck of E. Bilhuber, Inc., Orange, N. J. for solutions of the hydrochlorides of the other amines.

per kgm. and then 0.7, 1.0, or 1.4 mgm. amine hydrochloride per kgm. The animal was allowed to recover and the procedure repeated at one-week intervals with different amines until at least four experiments had been carried out in all animals. The epinephrine equivalence was estimated to the nearest 0.5 microgm. and the geometric mean equivalence calculated (see table 1).

Sections of jejunum of eight white rabbits were suspended in Tyrode solution at 37°C., and aerated with 95 per cent oxygen-5 per cent carbon dioxide. After various preliminary concentrations were tried, one mgm. of amine hydrochloride per 50 ml. tissue bath was chosen as a standard concentration. After two minutes exposure to the drug, the bath was flushed out three to five times. The responsiveness of the segments to 0.5 microgm. epinephrine base was used as a control.

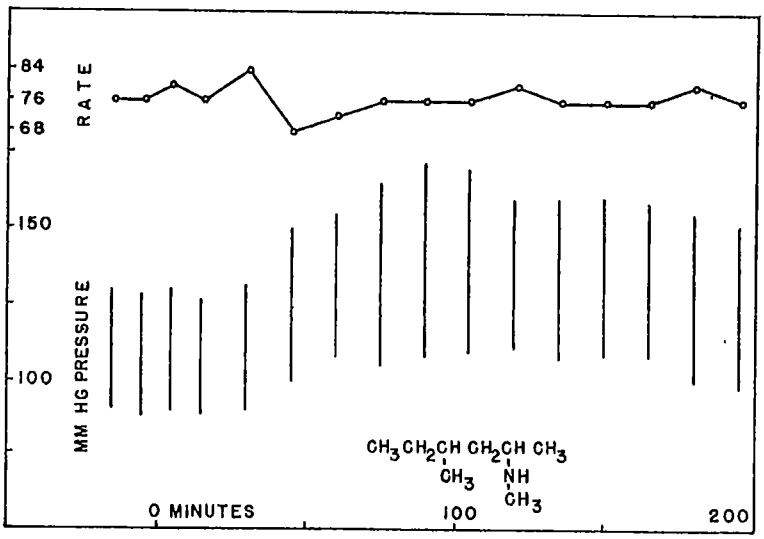

FIG. 1. Man (32 years old, 82 kgm.). Pulse rate above, systolic and diastolic blood pressure below measured by cuff sphygmomanometer. Three mgm. 4-methyl-2-hexyl methylamine hydrochloride per kgm. given orally in water at 0 time.

Mice were given the amine hydrochlorides intraperitoneally as 1 per cent solutions. Each mouse was kept in a separate pen at 22–23°C. Eight mice received each dose, and dose levels differed by 5 mgm./kgm. increments. All mice were observed four hours and the number dying in this time recorded. The data given in table 1 were all obtained from animals injected on a single day. The LD_{50}, to the nearest 5 mgm./kgm., was determined by the method of Litchfield and Wilcoxon (1949).

Five young adult males (22–32 years, 61–82 kgm.) were given 3 mgm. of the hydrochlorides of 4-methyl-2-hexyl methylamine, 4-methyl-2-hexylamine, 2-hexyl methylamine, and 2-hexylamine per kgm. orally with 200 ml. of water, four hours after a light morning meal. The agents were given at weekly intervals until all agents had been taken at least once. The systolic and diastolic blood pressure and the pulse rate were recorded every fifteen minutes for three hours while the subjects remained sitting quietly. Figure 1 is a typical plot of the data obtained.

RESULTS. The results of the animal experiments are summarized in table 1. Examination of the data indicates that replacement of one of the hydrogen

atoms on the amino nitrogen atom of the isomeric heptylamines does not markedly influence the vasopressor activity. Although some of the geometrical mean epinephrine equivalences are slightly greater or less for any pair of agents, the differences are not statistically significant. The responses of any given dog to the control doses of epinephrine varied as much as 18 per cent from week to week, although no dog had more than a 30 per cent differential variation in response to a given dose pair of epinephrine over the entire experimental period. In all four dogs in which the 4-methyl-2-hexyl and 2-heptyl pairs were crossed over, the 4-methyl-2-hexyl compounds were more active than the 2-heptyl type.

TABLE 1

NAME	PRESSOR ACTIVITY		RABBIT JEJUNUM—PERCENTAGE CHANGE IN AMPLITUDE PRODUCED BY 20 MGM./L.	MICE—I. P. LD_{50} MGM./KGM.
	Dose mgm./kgm. amine hydrochloride	Number of microgm. epinephrine/kgm. to have similar effect		
3-Heptylamine	1	0.5±	−20	90
3-Heptyl methylamine	1	0.5±	−50	70
2-Hexylamine	1	2.4	+100	60
2-Hexyl methylamine	1	2.1	+50	120
2-Methyl-2-hexylamine	1	1.0±	−50	85
	1.4	2.9		
2-Methyl-2-hexyl methylamine	1.4	3.0	−70	70
3-Methyl-2-hexylamine	1	2.0	−25	90
3-Methyl-2-hexyl methylamine	1	2.2	−60	70
4-Methyl-2-hexylamine	0.7	3.7	+40	185
	1	5.1		
4-Methyl-2-hexyl methylamine	1	4.6	−40	120
5-Methyl-2-hexylamine	1	3.2	−20	90
5-Methyl-2-hexyl methylamine	1	2.6	−70	65
2-Heptylamine	1	4.6	+70	95
2-Heptyl methylamine	1	4.2	+30	110

There was no obvious difference in duration of action between the methylated and unsubstituted amines.

Nitrogen methylation uniformly decreased the spasmogenic activity of a given compound if it produced an increase in amplitude of contraction of rabbit jejunum or further enhanced the relaxant effect if it produced a decrease in amplitude.

All the agents produce marked, irregular motor activity and convulsions in mice that received lethal doses. Pilomotor and exophthalmic responses are common. There is no obvious relationship between vasopressor effects in dogs, structure, presence or absence of a nitrogen methyl group, and lethal doses in mice.

About 45 minutes to one hour after the oral administration in man of 3 mgm. of one of the four more potent agents per kgm., the blood pressure begins to rise, the systolic-diastolic difference increases, and the pulse rate decreases. The

subjects complain of feeling hot or cold, that the skin tingles or itches, that there is a peculiar taste in the mouth or that the mouth is dry or the nose feels open. Pilomotor skin reactions are common. Mental confusion and inability to concentrate occasionally occur, although with no particular drug, and no evidence of central nervous stimulation as evidenced by excessive talkativeness is found. In four of the individuals the 4-methyl-2-hexyl methylamine is most potent in elevating the blood pressure, and in the other the 4-methyl-2-hexylamine is most active. The procedure was repeated to make certain no mistake had occurred in dosage or drug chosen. The results were the same within narrow limits. The individuals and the operator did not know which drug was given nor was the subject told the results of any measurements until after the experiment was completed.

Discussion. It seems fairly well established that nitrogen methyl substitution in the β-phenylalkylamine series decreases the vasopressor potency in dogs (Marsh, 1948a) as it does in the β-cyclohexylalkylamine series (Swanson and Chen, 1948; Marsh, 1948a) although it increases the activity of the β-cyclopentylalkylamines (Swanson and Chen, 1948). With this series of simple aliphatic amines, nitrogen methylation has little obvious effect on pressor potency. Regardless of the mechanism of action of these agents in producing this effect, apparently this chemical change influences neither absolute activity nor rate of disappearance. With oral doses in man of the potent members of this series, nitrogen methylation may actually influence the vasopressor response, and whether the nitrogen methyl substituted amine is more or less active than the unsubstituted amine may depend on the relative ability of the individual to absorb, transport, or eliminate (destroy or excrete) a particular type of agent.

The lack of relationship between structure and general toxicity or activity on isolated intestine is apparently a characteristic of the aliphatic amines, as it has been observed with related groups of agents (Marsh, 1948b; Marsh and Herring, 1950). However, other examples of nitrogen methylation enhancing the relative sympathomimetic depressant activity of amines on smooth muscle are known (Lands, 1949).

Although several publications on the activity of 2-heptyl methylamine have appeared (Ahlquist, 1944; Jackson, 1944; Roman-Vega and Adriani, 1946; Shaffer and Knoefel, 1950) and on the activity of 2-heptylamine and related compounds (Swanson and Chen, 1946; Marsh, 1948b) the various procedures have differed sufficiently that no comparison of the influence of nitrogen methylation is possible with the exception of the paper by Åhlquist who found 2-heptylamine to be more pressor and more toxic than 2-heptyl methylamine. None of the other isomeric heptyl methylamines seem to have been investigated. Both 2-heptylamine (Tuamine) and 4-methyl-2-hexylamine (Forthane) have been introduced as nasal vasoconstrictor agents (Chen, 1948) and 2-heptyl methylamine (Oenethyl) has been found to be a useful vasopressor substance for spinal anesthesia (Roman-Vega and Adriani, 1946). On the basis of the preliminary findings of our experiments, the 4-methyl-2-hexyl methylamine might prove equally useful for both of these purposes.

SUMMARY

1. The vasopressor activity of 3-heptyl, 2-methyl-2-hexyl, 3-methyl-2-hexyl, 4-methyl-2-hexyl, 5-methyl-2-hexyl, and 2-heptyl methylamine hydrochloride in dogs anesthetized with sodium thiopental and scopolamine has been determined and compared with the corresponding unsubstituted heptylamine hydrochloride and epinephrine. Nitrogen-methyl substitution does not significantly influence the vasopressor activity as compared with the unsubstituted amines and the compounds range from agents with very short duration of action and almost no pressor action to 4-methyl-2-hexyl methylamine which is about $\frac{1}{200}$ as active as epinephrine and has a long duration of action. The 2-heptyl methylamine is almost as active, and the corresponding unsubstituted amines may be slightly more active.

2. Nitrogen methylation of the isomeric heptylamines decreases the spasmogenic activity of the unsubstituted heptylamines on isolated rabbit jejunum or increases the relaxant activity.

3. All of the agents cause the death of white mice when given intraperitoneally in the dose range of 60–185 mgm./kgm. to individual mice kept at 22–23° C. and produce erection of hair, exophthalmos, increased motor activity and convulsions. Nitrogen methylation did not uniformly increase or decrease the toxicity of any given agent.

4. Oral doses of 3 mgm. of 4-methyl-2-hexyl methylamine, 4-methyl-2-hexylamine, 2-heptyl methylamine, and 2-heptylamine hydrochloride per kgm. produce marked vasopressor effects and have typical sympathomimetic actions. The 4-methyl-2-hexyl compounds were more active than the 2-heptyl derivatives.

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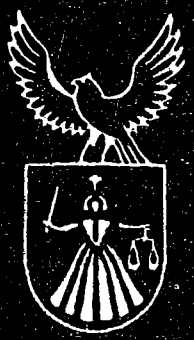
Arzneimittel-Forschung

drug research



des zerebralen Gefäßwiderstandes. Er schließt von der vermehrten Hirndurchblutung auf einen Anstieg des intrakraniellen Drucks. Zu einem ähnlichen Ergebnis kommt Dawson.

Die hirndrucksenkende Wirkung von Barbituratnarkosen hat Grote zusammenfassend beschrieben. Daher wurde in unseren Versuchen eine Narkose mit einem Barbiturat gewählt, um die Wirkung von Ketamin auf den intrakraniellen Druck nicht zu steigern.

Unsere Untersuchungen zeigen dennoch eine dosisabhängige Steigerung des intrakraniellen Drucks unter Ketamin. Der arterielle Druck sinkt im gleichen Zeitraum ab. Der zentralvenöse Druck verändert sich nicht in Abhängigkeit vom Medikament. Bekanntlich ändert sich der intrakranielle Druck bei Schwankungen des arteriellen Drucks innerhalb bestimmter Grenzen nicht, reagiert aber gegenüber venösen Druckschwankungen sehr empfindlich. Nach den vorliegenden Untersuchungen läßt sich daher nicht sicher sagen, welcher pathomechanische Effekt dieser nach Gaben von Ketamin beobachteten Zunahme des Hirndrucks zugrunde liegt. Eine Ödembildung kommt wegen der Flüchtigkeit der Drucksteigerung (5—20 min) nicht in Betracht.

Zusammenfassung

Untersucht wurde an 26 Kaninchen die Wechselwirkung zwischen arteriellem, zentralvenösem sowie intrakraniellem Druck. Die Ergebnisse anderer Autoren werden diskutiert, dennoch bleibt die pathogenetische Ursache für diese Beobachtungen offen, der Beweis, daß der intrakranielle Druck beim Kaninchen unter Ketamin (Ketanest®) dosisabhängig ansteigt (10, 15 und 20 mg/kg), konnte erbracht werden.

Summary

Intracranial Pressure and Circulatory Reactions to Ketamine in the Rabbit

A study has been designed to test the correlation of arterial, central-venous and intracranial pressure in 26 rabbits. Though the results of other authors are discussed, the pathogenetic cause of these observations cannot be clarified.

The results prove, however, that administration of ketamine (Ketanest®) increases the intracranial pressure of the rabbit in proportion to the applied dosage (10, 15, 20 mg/kg).

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Für die Verff.: Dr. Hans-Erik Clar, Neurochirurgische Universitätsklinik, 43 Essen, Hufelandstr. 55

From the Department of Pharmacology, University of Sydney, Sydney (Australia)

The Interaction between β-Phenylethylamine and Agents which Affect the Cholinergic Nervous System on Locomotor Activity and Toxicity in Mice

By D. M. Jackson

Introduction

β-Phenylethylamine (PE), a naturally occurring sympathomimetic amine (Nakajima, Kakimoto and Sano 1964; Jackson and Temple 1970) causes an increase in locomotor activity (LA) in mice (Schulte et al. 1941; Mantegazza and Riva 1963; Jackson 1972). The study of this material is important, because changes in endogenous levels of PE in man have been linked with cardiac disease (Jackson 1970), endogenous depression (Fischer, Heller and Miro 1968; Boulton and Milward 1971; Fischer et al. 1972) and phenylketonuria (Oates et al. 1963), and the suggestion has been made by Fischer, Saavedra and Heller (1968) that PE may play a role in the CNS as the ergotrophic substance of Hess (1959). Drugs which decrease cholinergic function like atropine and hyoscine potentiate many of the behavioural effects of amphetamine in mice and rats (Carlton and Didamo 1960; Carlton 1963; Mennear 1964). Because some of the pharmacological properties of PE resemble those of amphetamine (Jackson 1972; Jackson and Smythe 1973a), it was considered of interest as a means of determining the mode of action of PE to investigate its interaction with various cholinergically active drugs using locomotor activity and PE toxicity in mice.

Methods

QS strain male mice were used in all experiments. Except during experimentation mice were allowed food and water ad libitum and kept on a 12 h light, 12 h dark cycle. LA was measured in a circular actophotometer (30 cm × 30 cm high) intersected by four light beams, and the number of times the light beams were crossed was measured and automatically integrated and recorded on a 2 channel Grass polygraph. Groups of five animals were used in all LA studies. Mice were premedicated i.p. with a drug or vehicle control 30 min prior to an i.p. injection of either 50 or 100 mg PE/kg and then placed immediately in the activity cage for 1 h. Total LA was calculated between 20 and 60 min after injection, and the data presented as the number of times the light beams were cut. The data were analysed using the Student's t-test. The LA produced by the pretreatment plus PE combination was also expressed as a percentage of the pretreatment plus water combination (i.e., the control). Where correlation coefficients were calculated, the total activity of the pretreatment plus water group (i.e. the number of times the light beams were cut) was compared with the percentage as described in the latter sentence.

Studies on lethality were conducted with groups of ten mice in galvanised iron boxes of 11 cm × 6 cm × 5 cm height. In all cases studies were done between 2.00 and 5.00 p.m., in a quiet room. The number of dead animals was recorded 3 h after injection, and the data analysed using the method of Litchfield and Wilcoxon (1949). All drugs were dissolved in distilled water, in a dose-volume of 1 ml/100 g, and the doses calculated as the salts.

Origin of drugs

β-Phenylethylamine, methylhyoscine (Sigma), hyoscine hydrobromide (Burroughs Wellcome & Co.), physostigmine salicylate, atropine sulfate (Mc Farlane Smith), benzhexol hydrochloride (Lederle), benztropine mesylate (Merck, Sharp & Dohme), neostigmine bromide (Roche).

Results

PE produced a significant increase in LA in a concentration of 100 mg/kg, but not in a concentration of 50 mg/kg (Table 1), so these two doses were used in this study.

Benzhexol, benztropine, and atropine potentiated PE induced LA (Table 2), optimum doses being 2.5 mg/kg, 1 mg/kg and 5 mg/kg, respectively. Higher doses of these agents resulted in less potentiation accompanied by an increase in LA produced by the pretreatment alone.

Hyoscine, which readily crosses the blood-brain barrier, produced a dose-dependent increase in percent potentiation with both 50 and 100 mg PE/kg (Table 2). Maximum poten-

Table 1: Effect of 50 and 100 mg PE/kg on locomotor activity in mice. Mice were injected i.p. with PE or water, and activity measured between 20 and 60 min after injection. The number of experiments performed is in brackets, and the data presented is the mean number of times the light beams were cut during this period of time ± standard error (SEM). The value marked with an asterisk is significantly different ($P < 0.05$) from the water control.

Treatment	Total activity ± SEM	Percent of control**)
Water	871 ± 116 (20)	100
50 mg PE/kg	1169 ± 101 (18)	134
100 mg PE/kg	1742 ± 107 (18)*)	200

**) compared to water as 100%

tiation occurred with a dose of 1 mg hyoscine/kg. Methylhyoscine which is much less effective than hyoscine in crossing the blood-brain barrier, produced a trough-like effect on percent potentiation when combined with 50 and 100 mg PE/kg (Table 2). Maximum and significant potentiation occurred with doses of 0.01 and 1.0 mg methylhyoscine/kg, but not with doses of 0.1 and 0.5 mg/kg. A correlation coefficient between total activity of the pretreatment control itself and the percentage potentiation of LA with PE 50 and 100 mg/kg showed a negative correlation with methylhyoscine (Table 2).

A significant correlation was not obtained where atropine, benzhexol, benztropine and hyoscine were the pretreatments except in one instance where benzhexol was combined with 100 mg PE/kg (Table 2).

Table 2: The effect of various premedications on β-phenylethylamine induced locomotor activity in mice. All premedications were administered i.p. 30 min prior to PE or water.

The total number of times groups of five mice cut the light beams in an actophotometer was measured between 20 and 60 min after the second injection and expressed as the mean ± SEM (number of experiments in brackets). Each results was also expressed as a percent of the control value (100%). A correlation co-efficient (r) was calculated between the percent potentiation for 50 and 100 mg PE/kg and the total activity for the pretreatment by itself and the probability (P) calculated. The values marked with an asterisk are significantly different ($P < 0.05$) from the appropriate pretreatment plus water control.

Dose of PE (mg/kg)	Locomotor activity ± SEM	Percent of control	Locomotor activity ± SEM	Percent of control	Locomotor activity ± SEM	Percent of control	Locomotor activity ± SEM	Percent of control	r	P
	Atropine									
	1 mg/kg		2 mg/kg		5 mg/kg		10 mg/kg			
water	790 ± 185 (7)	100	602 ± 199 (6)	100	458 ± 232 (5)	100	1279 ± 278 (7)	100		
50	940 ± 108 (7)	119	936 ± 90 (6)	155	1399 ± 169 (5)	305*)	2051 ± 337 (6)	160	—0.5259	>0.1
100	1062 ± 68 (7)	134	1464 ± 171 (6)	243	1603 ± 227 (5)	350*)	2975 ± 90 (6)	233*)	—0.4271	>0.1
	Benzhexol									
	0.5 mg/kg		1.0 mg/kg		2.5 mg/kg		10.0 mg/kg			
water	770 ± 238 (7)	100	673 ± 214 (7)	100	473 ± 98 (5)	100	1285 ± 262 (6)	100		
50	669 ± 43 (7)	87	1223 ± 85 (7)	182	1529 ± 116 (5)	323*)	2370 ± 257 (5)	184*)	—0.4193	>0.1
100	1522 ± 89 (7)	198*)	1786 ± 94 (6)	265*)	2423 ± 179 (5)	512*)	2273 ± 106 (5)	177*)	—0.7599	>0.025 <0.05
	Benztropine									
	0.5 mg/kg		1.0 mg/kg		2.0 mg/kg					
water	768 ± 208 (6)	100	825 ± 123 (6)	100	1724 ± 409 (7)	100				
50	1094 ± 107 (6)	142	1912 ± 195 (6)	232*)	2230 ± 266 (7)	129			—0.5535	>0.1
100	1344 ± 117 (6)	175*)	2310 ± 74 (6)	280*)	2177 ± 173 (7)	126			—0.7083	>0.1
	Hyoscine									
	0.01 mg/kg		0.1 mg/kg		0.5 mg/kg		1.0 mg/kg			
water	695 ± 194 (8)	100	367 ± 105 (6)	100	745 ± 255 (6)	100	596 ± 118 (7)	100		
50	878 ± 119 (7)	126	856 ± 116 (6)	233*)	2078 ± 240 (7)	279*)	2307 ± 260 (7)	387*)	—0.0768	>0.1
100	1419 ± 73 (6)	204*)	1416 ± 175 (6)	386*)	2126 ± 173 (7)	285*)	2453 ± 178 (6)	412*)	—0.6613	>0.1
	Methylhyoscine									
	0.01 mg/kg		0.1 mg/kg		0.5 mg/kg		1.0 mg/kg			
water	233 ± 64 (7)	100	421 ± 127 (7)	100	659 ± 209 (7)	100	230 ± 68 (7)	100		
50	757 ± 100 (7)	325*)	769 ± 121 (7)	183	1099 ± 174 (7)	167	645 ± 157 (6)	280*)	—0.8882	0.001—0.01
100	1452 ± 141 (7)	623*)	1233 ± 121 (7)	293*)	1563 ± 224 (6)	237*)	1250 ± 81 (7)	543*)	—0.9151	0.001—0.01
	Physostigmine									
	4.25 μg/kg		8.5 μg/kg		17 μg/kg		34 μg/kg			
water	718 ± 202 (8)	100	510 ± 185 (8)	100	376 ± 89 (6)	100	411 ± 136 (7)	100		
50	1325 ± 179 (7)	185*)	968 ± 163 (7)	190	933 ± 100 (8)	248*)	1056 ± 127 (7)	257*)	—0.9263	<0.001
100	1570 ± 90 (7)	219*)	1616 ± 107 (6)	317*)	1455 ± 89 (6)	387*)	1841 ± 216 (7)	448*)	—0.7392	0.001—0.01
	68 μg/kg		136 μg/kg		250 μg/kg		500 μg/kg			
water	384 ± 85 (7)	100	334 ± 109 (6)	100	407 ± 107 (7)	100	1014 ± 210 (6)	100		
50	848 ± 172 (7)	221*)	742 ± 85 (6)	222*)	855 ± 94 (8)	210*)	696 ± 109 (6)	69		
100	1703 ± 55 (7)	443*)	1423 ± 117 (6)	426*)	955 ± 75 (8)	235*)	2115 ± 217 (6)	209*)		
	Neostigmine									
	6.25 μg/kg		12.5 μg/kg		25 μg/kg		50 μg/kg			
water	652 ± 199 (7)	100	152 ± 24 (7)	100	451 ± 156 (7)	100	384 ± 115 (7)	100		
50	950 ± 144 (6)	146	1015 ± 127 (7)	668*)	1256 ± 327 (6)	278*)	835 ± 249 (5)	217	—0.920	<0.001
100	1821 ± 247 (6)	279*)	1444 ± 126 (7)	950*)	1468 ± 118 (6)	325*)	1589 ± 232 (5)	414*)	—0.9043	<0.001
	100 μg/kg		184 μg/kg							
water	451 ± 88 (6)	100	749 ± 233 (7)	100						
50	1467 ± 260 (6)	325*)	546 ± 94 (7)	73						
100	1793 ± 164 (6)	398*)	1429 ± 81 (7)	191*)						

Table 3: Effect of 30 min pretreatment with hyoscine (100 or 1000 μg/kg) combined with physostigmine (34 or 250 μg/kg) on LA produced by 50 and 100 mg PE/kg. The results are expressed as the total number of counts between 20 and 60 min after injection and as the percent increase in LA after a dose of PE compared to control levels (as 100%). The number of experiments is in brackets and the SEM is given. The values marked with an asterisk are significantly different ($P < 0.05$) from the appropriate water control.

	Pretreatment (1st dose)			
	Hyoscine 100 μg/kg		Hyoscine 1000 μg/kg	
2nd dose	Physostigmine 34 μg/kg	Physostigmine 250 μg/kg	Physostigmine 34 μg/kg	Physostigmine 250 μg/kg
Distilled water	348 ± 82 (7) 100%	273 ± 93 (7) 100%	954 ± 277 (7) 100%	1022 ± 92 (7) 100%
PE 50 mg/kg	867 ± 175*) (7) 249%	761 ± 171*) (7) 279%	2012 ± 237*) (6) 211%	1975 ± 152*) (7) 193%
PE 100 mg/kg	1353 ± 155*) (7) 389%	1275 ± 111*) (7) 467%	1726 ± 158*) (7) 181%	2020 ± 223*) (6) 198%

*) $P < 0.05$

Table 4: Effect of pretreatment with either hyoscine or physostigmine on PE toxicity in mice. The data were analysed using the method of Litchfield and Wilcoxon (1949). The number of animals (n) used is in brackets. For further details see methods.

Pretreatment	LD_{50} (mg/kg) (95% limit)	Slope function (fiducial limits)	n
Hyoscine 500 μg/kg	112 (82—152)*)	1.7455 (1.25—2.44)	380
Physostigmine 250 μg/kg	155 (144—167)	1.5114 (1.33—1.72)	280
None	175 (146—209)	1.8737 (1.29—2.72)	270

*) $P < 0.05$

Physostigmine and neostigmine were tested in doses between 4.25 and 500 μg/kg and 6.25 and 368 μg/kg, respectively. Since a dose of 368 μg/kg of neostigmine had been lethal in 4 of 10 animals in preliminary experiments, this dose was not further used. With results expressed as a percentage, both drugs produced significant potentiation (Table 2). Maximum potentiation of both doses of PE occurred with 12.5 μg/kg neostigmine and 34 μg physostigmine/kg. Higher doses of both of these drugs caused a reduction in percent potentiation. A highly significant negative correlation was noted (Table 2) between the effect of both the drugs by themselves on LA, and the respective percentage potentiations when combined with PE.

Since 1 mg hyoscine/kg produced a marked potentiation, this dose was combined with either 34 or 250 μg physostigmine/kg. Table 3 shows that both doses of physostigmine reduced the percent potentiation of PE observed with hyoscine 1 mg/kg only, (cf. Table 2) but significant potentiation was still observed. When hyoscine 100 μg/kg was combined with both these doses of physostigmine, the potentiation with 50 or 100 mg PE/kg was virtually identical to that observed with 100 μg hyoscine/kg alone (Table 2).

The LD_{50} of PE for aggregated mice was 175 mg/kg, which was significantly more than the LD_{50} of the hyoscine pretreated group (Table 4). When physostigmine pretreatment was used, there was no significant change in LD_{50} from that of PE alone.

Discussion

Although PE differs from amphetamine in producing a biphasic locomotor response in mice (Jackson 1972) and in having a very short duration of action because of its rapid metabolism by monoamine oxidase (Blaschko 1952), the present study in mice shows that agents which block the muscarinic actions of acetylcholine potentiate PE induced LA. In this regard, PE resembles both amphetamine (Carlton 1961), and the weak sympathomimetic phenylpropanolamine (Davis and Pinkerton 1972). Preliminary inspection of the data obtained with methylhyoscine, physostigmine and neostigmine suggested that these agents, as well as hyoscine, atropine, benztropine and benzhexol, produced a potentiation of the effect of PE on LA in mice. However, when the percent potentiations of both doses of PE by the former three pretreatments were correlated with their respective control values (i.e. pretreatment and water only), it became evident that the potentiation in each case was significantly correlated with the effect each pretreatment alone produced. This significant correlation was not seen (except in one case) with pretreatments using atropine- hyoscine, benzhexol and benztropine. Thus it appears that the apparent percent potentiation with physostigmine, neostigmine and methylhyoscine is a result of the depressant effect of the pretreatment itself, rather than a real interaction between the pretreatment and PE. Since there was no significant correlation obtained with hyoscine, atropine, benztropine and benzhexol, it is further suggested that the potentiation of PE induced LA in each of these cases is in fact due to an interaction between the pretreatment and PE. In this case the potentiation of PE is independent of any depressant or stimulant effect the pretreatment may have by itself. The increase in lethality when hyoscine pretreatment was used confirms the ability of cholinergic blockers to augment the effect of PE specifically, and sympathomimetics in general (Davis and Pinkerton 1972).

The potentiation by atropine, hyoscine, benztropine and benzhexol but not by methylhyoscine suggests that the potentiation is centrally mediated rather than peripherally mediated, confirming earlier conclusions obtained with amphetamine (Carlton 1961; Carlton and Didamo 1961).

Because of the apparent potentiation of LA by pretreatment with physostigmine and neostigmine, a conclusion cannot be drawn as to whether these agents actually antagonise the increased LA produced by PE. However, when physostigmine was combined with 100 μg hyoscine/kg, the effects were not additive, and the percent potentiation was similar to that produced by hyoscine plus PE only. Only with a dose of 1 mg hyoscine/kg was there a reduction of physostigmine of PE induced LA. Physostigmine, in a dose equal to half of its LD_{50}, failed to protect the mice against PE toxicity, suggesting that physostigmine will not antagonise the effects of PE. These results contrast with those of Goldberg and Ciofalo (1969), who found that physostigmine reversed the action of amphetamine on a non-discriminated avoidance schedule in rats. The discrepancy here is probably due to species and parameter differences.

The mechanism of action of these agents is unclear. However some anticholinergic agents are able to block dopamine (DA) uptake into the corpus striatum (Coyle and Snyder 1969; Farnebo et al. 1970) and are able to cause changes in cerebral dopamine turnover (Bartholini and Pletscher 1971; Corrodi et al. 1967). There is also a possible cholinergic mechanism in the caudate nucleus involved in motor conditioned responses (Prado-Alcata et al. 1972). It is possible therefore that this interaction with DA neurons is the basis of the cholinergic interaction with PE, since it has been shown that the distribution of PE in the CNS is intimately related to that of DA (Jackson and Smythe 1973b), and that agents which make more DA available in the CNS, potentiate the effect of PE on LA in mice (Jackson, unpublished observation).

Summary

This paper describes the effect of a combination of β-phenylethylamine together with hyoscine, methylhyoscine, physostigmine, atropine, benzhexol, benztropine or neostigmine on locomotor activity in mice. The locomotor activity induced by 100 mg phenylethylamine/kg was potentiated by hyoscine, atropine, benztropine and benzhexol, all of which drugs readily penetrate the CNS, but not by methylhyoscine, a drug which does not readily cross the CNS. Neither physostigmine and neostigmine, revealed a clear potentiating or inhibiting effect on phenylethylamine induced locomotor activity. The lethality of phenylethylamine in mice was studied alone (LD_{50} 175 mg/kg), combined with hyoscine (112 mg/kg) or with physostigmine (155 mg/kg). Hyoscine significantly potentiates the lethality of phenylethylamine. The possibility is considered that the interaction of these agents with phenylethylamine may involve dopamine.

Zusammenfassung

Wechselwirkungen zwischen β-Phenyläthylamin und Stoffen mit Wirkung auf das cholinerge Nervensystem und ihr Einfluß ouf Lokomotoraktivität und Toxizität bei der Maus

Die Wirkung der Kombination von β-Phenyläthylamin mit Hyoscin, Methylhyoscin, Physostigmin, Atropin, Benzhexol, Benztropin oder Neostigmin auf die Lokomotoraktivität von Mäusen wird beschrieben. Die durch 100 mg Phenyläthylamin/kg induzierte Lokomotoraktivität wurde durch Hyoscin, Atropin, Benztropin und Benzhexol verstärkt. All diese Substanzen penetrieren leicht in das Zentralnervensystem, nicht aber Methylhyoscin. Weder Physostigmin noch Neostigmin zeigten einen eindeutig verstärkenden oder hemmenden Effekt auf die durch Phenyläthylamin induzierte Lokomotoraktivität. Die letale Wirkung von Phenyläthylamin auf Mäuse wurde allein untersucht (LD_{50} 175 mg/kg) wie auch in Kombination mit Hyoscin (112 mg/kg) oder Physostigmin (155 mg/kg). Hyoscin erhöht die letale Wirkung von Phenyläthylamin signifikant. Es wird die Möglichkeit in Betracht gezogen, daß die Wechselwirkungen dieser Substanzen mit Phenyläthylamin auch Dopamin miteinbeziehen.

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Address of the author: Dr. D. M. Jackson, Department of Pharmacology, University of Sydney, Sidney, N.S.W. 2006 (Australia)

From the (former) Research Department[1]) of the Laboratoires Houdé, Paris, and the Faculty of Medicine[2]), University of Nancy (France)

Studies on the Effects of Piridoxilate, a Glyoxylic Acid Derivative, on the Mammalian System, Heart and Muscle under Normal or Deficient Oxygen Supply

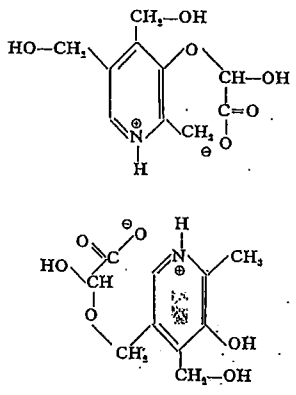
Attempts towards a biochemical approach

By J. P. Fourneau[1]), M. Davy[1]), M. Clément[1]), M. Ranson[1]), F. Darmois[1]) and M. Lamarche[2])

1.0. Introduction

One of our team [1] has elsewhere set out the considerations which led him, with vitamin B_{15} as the starting point, to suggest the therapeutical utilisation of glyoxylic acid. This suggestion was essentially based on a hypothesis put out by Ruffo [2, 3]: glyoxylic acid would seem to be a physiological regulator of cell respiration liable to limit cell oxygen consumption strictly to oxydation processes coupled with ATP-producing phosphorylations.

In order to keep down, as much as possible, its transformation into oxalic acid in the system, glyoxylic acid has been combined with pyridoxine: for this substance, as a precursor of vitamin B_6, cofactor of transaminases and decarboxylases, should direct almost its entire metabolism towards transamination into glycine and decarboxylation into formic acid, at the expense of its oxidation into oxalic acid. Piridoxilate is the result of this combination, in the form of the reciprocal salt of two isomeric hemiacetals [4]:



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Pyrimidine Derivatives and Related Compounds. XXII.[1] Synthesis and Pharmacological Properties of 7-Deazaxanthine Derivatives

Shigeo Senda and Kosaku Hirota

Gifu College of Pharmacy[2]

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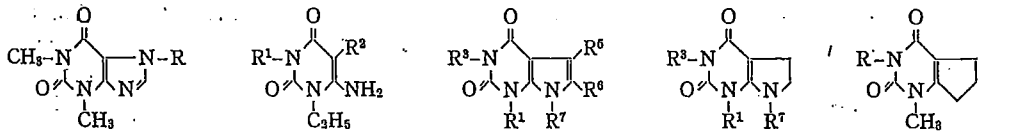
For investigation of the structure-activity relationship of xanthine derivatives, 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines (A), which belong to 7-deazaxanthine derivatives, were prepared from the corresponding 6-aminouracils and chloroacetaldehyde, and then were catalytically reduced to give 2,4-dioxo-1,2,3,4,5,6-hexahydropyrrolo[2,3-*d*]-pyrimidines (B). A new method for synthesis of compounds (A) was found by heating 6-hydrazinouracil derivatives with aldehydes or ketones.

Diuretic, cardiac, and central nervous system stimulating activities of compounds (A and B) were tested to be compared with those of caffeine. Compounds (A and B) showed caffeine-like activities.

Xanthine derivatives such as caffeine (**1**) and theophylline (**2**) have been used as a diuretic cardiotonica for a long time. 6-Aminouracil derivatives such as 3-allyl-6-amino-1-ethyluracil (**3**: Aminometradine)[3] and 6-amino-1,3-diethyl-5-isopropyluracil (**4**)[4] had once been used as a synthetic diuretic drug.

In the present paper, 7-deazaxanthines such as 2,4-dioxo-1,2,3,4-tetrahydropyrrolo-[2,3-*d*]pyrimidines (A) and 2,4-dioxo-1,2,3,4,5,6-hexahydropyrrolo[2,3-*d*]pyrimidines (B) were synthesized and their diuretic, cardiac, and central nervous system (CNS) stimulating activities were tested to investigate their structure-activity relationship. It is because theoretically the chemical structares of A are derived by a replacement of the nitrogen atom at 7-position of xanthine ring with a methine group and those of B are derived by a ring-closure between 6-amino group and 5-alkyl group of 5-alkyl-6-aminouracil compounds.

In this connection, diuretic, cardiac, and CNS stimulating activities of 2,4-dioxo-1,2,3,4,6,7-hexahydro-5H-cyclopenta[*d*]pyrimidines (C),[5] namely, those of 7,9-dideaza-8,9-dihydroxanthine derivatives, were also tested in order to research a role of the nitrogen atom at 9-position of 7-deazaxanthines and the 6-amino group of uracils on their pharmacological properties.



1: R=CH_3 (caffeine) 3: R^1=$CH_2CH{=}CH_2$, R^2=H A B C

2: R=H (theophylline) (aminometradine) 94: R=CH_3

4: R^1=C_2H_5, R^2=iso-Pr 95: R=C_6H_5

96: R= 

Chart 1

1) Part XXI: S. Senda, K. Hirota, and O. Otani, *Yakugaku Zasshi*, 94, 571 (1974).
2) Location: *492-36, Mitahora, Gifu.*
3) V. Papesch and E.F. Schroeder, *J. Org. Chem.*, 16, 1879 (1951).
4) W. Stoll, Ger. Patent 938846 (1956) [*Chem. Abstr.*, 53, 6273 (1959)]; W. Stoll, Jap. Patent 244973 (1958).
5) S. Senda, K. Hirota, and K. Maeno, *Chem. Pharm. Bull.* (Tokyo), 21, 1894 (1973).

Chemistry

Up to now, some syntheses of 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines or 7-deazaxanthines have been reported.[6] According to the method of Noell and Robins,[6b] 6-aminouracils (I) were heated with chloroacetaldehyde in the presence of sodium acetate so that a ring closure took place to give 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines (II: **5**—**20** in Table I). Methylation of II with dimethyl sulfate in an aqueous solution of sodium hydroxide gave 7-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines (III: **21**—**27** in Table I). When these pyrrolo[2,3-*d*]pyrimidine derivatives (II and III) were reduced in ethanol at 50—60 atm and 100—120° in the presence of Pd-C, 2,4-dioxo-1,2,3,4,5,6-hexahydropyrrolo[2,3-*d*]pyrimidines (IV: **28**—**42** in Table II) were prepared. The 1-allyl groups of **13** and **14** were further reduced by the above mentioned catalytic reduction to give the corresponding 1-propyl compounds (**34**, **35**). 1-Benzyl-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidine (**17**) was reduced and debenzylated under the same conditions described above to yield **29**.

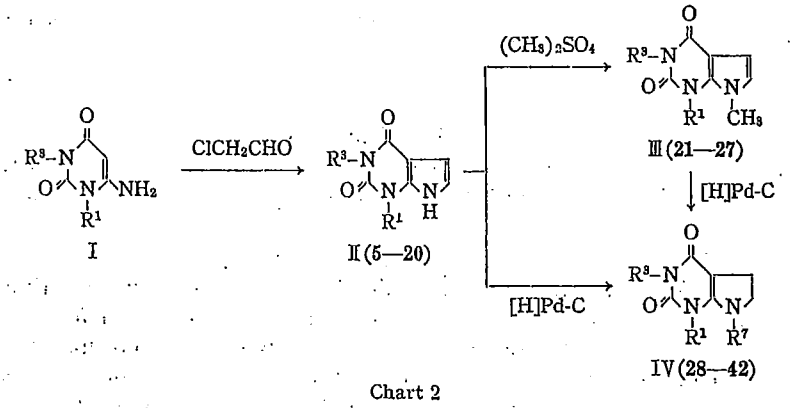


Chart 2

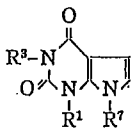
Then the authors investigated[7] a new method for synthesis of 5-substituted or 5,6-disubstituted 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidine derivatives (VIII) by heating 6-hydrazinouracil derivatives (V: **43**—**46**) with aldehydes or ketones. Thus 1,3-disubstituted 6-hydrazinouracil (V) was refluxed in ethanol or xylene with acetaldehyde, propionaldehyde, butyraldehyde, valeraldehyde or methyl ethyl ketone and the resulting hydrazones (VI: **47**—**59** in Table III) were refluxed for 2—3 hours in ethylene glycol or tetraline to give 5,6-disubstituted 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines (VII: **60**—**64**, **76**, **82** in Table IV) with an evolution of ammonia (Method A). When 6-hydrazinouracil derivatives (V: **43**—**46**) were refluxed in ethylene glycol or tetraline with aldehydes or ketones, the desired products (VII: **63**—**90** in Table IV) were obtained without isolation of the intermediates (VI) (Method B).

Pyrrolo[2,3-*d*]pyrimidines (**89**, **90**) having a ethoxycarbonylmethyl group at 5-position were hydrolyzed in an aqueous solution of sodium hydroxide to give 5-carboxymethyl derivatives (**91**, **92**), and the hydrolysis of **89** in hydrochloric acid gave the decarboxylated compound (**64**).

6) *a*) R.K. Robins and G.H. Hitchings, Brit. Patent 812366 (1959) [*Chem. Abstr.*, 54, 592 (1960)]; *b*) C.W. Noell and R.K. Robins, *J. Heterocycl. Chem.*, 1, 34 (1964); *c*) E.C. Taylor and E.E. Garcia, *J. Org. Chem.*, 30, 655 (1965); *d*) H. Ogura, M. Sakaguchi, and K. Takeda, *Chem. Pharm. Bull.* (Tokyo), 20, 404 (1972).

7) A part of this work has been reported in a communication: S. Senda and K. Hirota, *Chemistry Lett.*, 1972, 367.

TABLE I. 1,3,7-Trisubstituted 2,4-Dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines

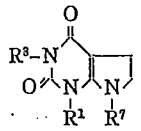


Compd. No.	R^1	R^3	R^7	mp (°C) Recryst. solvent	Yield (%)	UV λ_{max}^{EtOH} mμ (ε×10^{-3})	Formula		Analysis (%) C	H	N
5	H	H	H	>300[a] DMF	53	[b]	$C_6H_5O_2N_3$	Calcd.	47.68	3.34	27.81
								Found	47.44	3.51	27.49
6	Me	Me	H	300[c] H_2O	54	243(6.7) 275(6.7)	$C_8H_9O_2N_3$	Calcd.	53.62	5.06	23.45
								Found	53.89	5.22	23.68
7	Et	H	H	>300 H_2O	65	241(7.5) 274(6.7)	$C_8H_9O_2N_3$	Calcd.	53.62	5.06	23.45
								Found	53.69	5.28	23.30
8	Et	Me	H	234—236 H_2O	56	243(6.9) 274(6.9)	$C_9H_{11}O_2N_3$	Calcd.	55.95	5.74	21.75
								Found	56.03	5.81	21.97
9	Et	Et	H	203 H_2O	58	243(7.7) 275(7.5)	$C_{10}H_{13}O_2N_3$	Calcd.	57.96	6.32	20.28
								Found	57.86	6.38	20.44
10	*n*-Pr	H	H	295 EtOH	70	241(8.1) 274(7.4)	$C_9H_{11}O_2N_3$	Calcd.	55.95	5.74	21.75
								Found	56.02	5.71	21.64
11	*n*-Pr	Me	H	207—209 H_2O	65	243(6.0) 274(6.1)	$C_{10}H_{13}O_2N_3$	Calcd.	57.96	6.32	20.28
								Found	58.07	6.13	20.55
12[d]	*n*-Pr	*n*-Pr	H								
13	$CH_2CH=CH_2$	H	H	>300 EtOH	58	241(6.4) 274(6.3)	$C_9H_9O_2N_3$	Calcd.	56.54	4.75	21.98
								Found	56.71	4.89	21.86
14	$CH_2CH=CH_2$	Me	H	219 AcOEt	51	243(6.9) 274(7.4)	$C_{10}H_{11}O_2N_3$	Calcd.	58.53	5.40	20.48
								Found	58.79	5.69	20.40
15	*n*-Bu	H	H	275 EtOH–H_2O	11	241(7.2) 275(6.6)	$C_{10}H_{13}O_2N_3 \cdot H_2O$[e]	Calcd.	53.32	6.71	18.66
								Found	53.22	6.66	19.11
16	*n*-Bu	Me	H	165 AcOEt	17	243(6.2) 274(6.3)	$C_{11}H_{15}O_2N_3$	Calcd.	59.71	6.83	18.99
								Found	59.99	6.79	18.76
17	CH_2Ph	Me	H	196 EtOH–H_2O	42	244(6.1) 276(7.4)	$C_{14}H_{13}O_2N_3$	Calcd.	65.87	5.13	16.42
								Found	66.04	5.38	16.68
18	Ph	H	H	>300 EtOH–H_2O	36	242(6.7) 276(6.5)	$C_{12}H_9O_2N_3$	Calcd.	63.43	3.99	18.49
								Found	63.30	4.38	18.04
19	Ph	Me	H	280 MeOH–H_2O	82	240(5.0) 278(6.4)	$C_{13}H_{11}O_2N_3$	Calcd.	64.72	4.60	17.43
								Found	64.74	4.79	17.63
20	*p*-MePh	Me	H	235 H_2O	31	239(7.4) 278(9.1)	$C_{14}H_{13}O_2N_3$	Calcd.	65.87	5.13	16.46
								Found	65.51	5.32	16.22
21	Me	Me	Me	265—266 EtOH	68	247(5.5) 277(5.1)	$C_9H_{11}O_2N_3$	Calcd.	55.95	5.74	21.75
								Found	55.91	5.74	21.90
22	Et	Me	Me	215—217 H_2O	75	248(7.4) 277(6.8)	$C_{10}H_{13}O_2N_3$	Calcd.	57.96	6.32	20.28
								Found	57.99	6.45	20.43
23	Et	Et	Me	160—163 H_2O	71	248(7.3) 277(6.8)	$C_{11}H_{15}O_2N_3$	Calcd.	59.71	6.83	18.99
								Found	59.81	7.03	19.02
24	$CH_2CH=CH_2$	Me	Me	162 H_2O	78	248(6.4) 277(6.3)	$C_{11}H_{13}O_2N_3$	Calcd.	60.26	5.98	19.15
								Found	60.35	6.10	19.13
25	CH_2Ph	Me	Me	190—191 EtOH–H_2O	69	247(6.0) 278(6.6)	$C_{15}H_{15}O_2N_3$	Calcd.	66.90	5.61	15.61
								Found	66.62	5.88	15.66
26	Ph	Me	Me	277 MeOH	74	246(5.2) 279(5.8)	$C_{14}H_{13}O_2N_3$	Calcd.	65.87	5.13	16.46
								Found	65.95	5.34	16.50
27	*p*-MePh	Me	Me	276 EtOH–H_2O	77	243(7.2) 278(8.4)	$C_{15}H_{15}O_2N_3$	Calcd.	66.90	5.61	15.61
								Found	66.97	5.83	15.74

Ph=phenyl

a) lit.[6a] mp 330°. b) J. Davoll, *J. Chem. Soc.*, 1960, 131, UV $\lambda_{max}^{H_2O\,(pH\,6.8)}$ mμ (ε×10^{-3}): 243 (7.1), 275 (6.3) c) lit.[4b] mp 292—294° d) This compound was not purified and reduced directly to give 36 (in Table II). e) H_2O was confirmed by IR spectra.

TABLE II. 1,3,7-Trisubstituted 2,4-Dioxo-1,2,3,4,5,6-hexahydropyrrolo[2,3-*d*]pyrimidines



Compd. No.	R^1	R^3	R^7	mp (°C) Recryst. solvent	Yield (%)	UV λ_{max}^{EtOH} mμ (ε×10^{-3})	Formula		Analysis (%) C	H	N
28	H	H	H	>300[a] EtOH	13	239(5.3) 280(12.2)	$C_6H_7O_2N_3$	Calcd.	47.05	4.61	27.44
								Found	46.83	4.59	27.11
29	H	Me	H	285—286 H_2O	60	243(1.5) 282(13.0)	$C_7H_9O_2N_3$ H_2O[b]	Calcd.	45.40	5.99	22.65
								Found	45.33	5.96	22.68
30	Me	Me	H	283 EtOH	66	243(2.1) 284(17.9)	$C_8H_{11}O_2N_3$	Calcd.	53.03	6.12	23.19
								Found	53.09	6.21	23.08
31	Et	H	H	>300 H_2O	50	236(3.2) 284(17.8)	$C_8H_{11}O_2N_3$	Calcd.	53.03	6.12	23.19
								Found	53.25	6.31	23.33
32	Et	Me	H	258 H_2O	74	243(2.0) 284(17.2)	$C_9H_{13}O_2N_3$	Calcd.	55.37	6.71	21.53
								Found	55.61	6.49	21.66
33	Et	Et	H	191—192 H_2O	64	240(2.6) 285(17.5)	$C_{10}H_{15}O_2N_3$	Calcd.	57.40	7.23	20.08
								Found	57.69	7.30	20.18
34	*n*-Pr	H	H	285—287 H_2O	51 (65)[c]	236(2.8) 285(16.8)	$C_9H_{13}O_2N_3$	Calcd.	55.37	6.71	21.53
								Found	55.24	6.68	21.56
35	*n*-Pr	Me	H	181—182 H_2O	60 (65)[d]	238(2.8) 284(17.8)	$C_{10}H_{15}O_2N_3$	Calcd.	57.40	7.23	20.08
								Found	57.34	7.07	20.29
36	*n*-Pr	*n*-Pr	H	170—172 AcOEt	26	244(2.0) 286(16.8)	$C_{12}H_{19}O_2N_3$	Calcd.	60.73	8.02	17.71
								Found	60.82	8.04	17.65
37	*n*-Bu	H	H	243—244 H_2O	76	233(2.4) 283(16.5)	$C_{10}H_{15}O_2N_3 \cdot 1/2H_2O$[b]	Calcd.	55.03	7.39	19.25
								Found	55.02	7.36	19.10
38	*n*-Bu	Me	H	151—152 H_2O	76	243(1.9) 285(15.4)	$C_{11}H_{17}O_2N_3$	Calcd.	59.17	7.68	18.82
								Found	59.48	7.87	18.55
39	Ph	H	H	292—293 H_2O	74	287(19.4)	$C_{12}H_{11}O_2N_3$	Calcd.	62.87	4.84	18.33
								Found	62.86	5.04	18.31
40	Ph	Me	H	270—272 EtOH	75	287(17.8)	$C_{13}H_{13}O_2N_3$	Calcd.	64.18	5.39	17.28
								Found	64.15	5.21	17.36
41	*p*-MePh	Me	H	228—229 H_2O	40	286(19.9)	$C_{14}H_{15}O_2N_3$	Calcd.	65.35	5.88	16.33
								Found	65.36	5.94	16.53
42	Ph	Me	Me	205 H_2O	60	298(17.8)	$C_{14}H_{15}O_2N_3$	Calcd.	65.35	5.88	16.33
								Found	65.30	5.74	16.54

Ph=phenyl

a) V. G. Granik and R. G. Glushkov, *Khim.-Farm. Zh.*, **1** (5), 16 (1967) [*Chem. Abstr.*, **68**, 12941 (1968)], mp >300°
b) H_2O was confirmed by IR spectra. *c*) prepared from **13** *d*) prepared from **14**

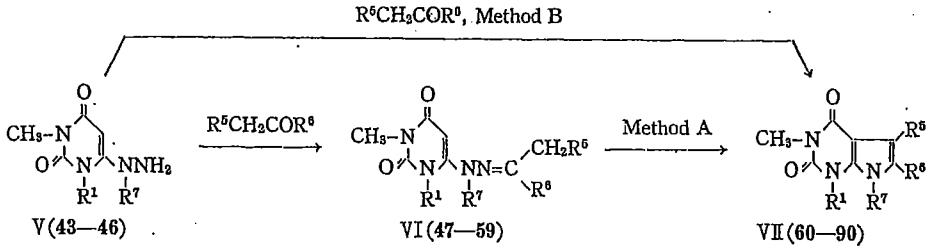
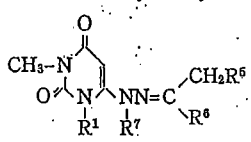


Chart 3

The 5,6-double bond of such 5,6-disubstituted 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]-pyrimidines (VII) were generally resistant to catalytic reductions except **64** which was reduced to give 1,3,5,6-tetramethyl-2,4-dioxo-1,2,3,4,5,6-hexahydropyrrolo[2,3-*d*]pyrimidine (**93**).

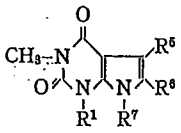
TABLE III. 1-Substituted 6-Alkylenehydrazino-3-methyluracils



Compd. No.	R^1	R^5	R^6	R^7	mp (°C) Recryst. solvent	Yield (%)	Formula		Analysis (%) C	H	N
47	Me	Me	H	H	203 EtOH	86	$C_9H_{14}O_2N_4$	Calcd.	51.42	6.71	26.65
								Found	51.26	6.84	26.55
48	Me	Et	H	H	179 EtOH	83	$C_{10}H_{16}O_2N_4$	Calcd.	53.55	7.16	24.99
								Found	53.34	6.96	25.17
49	Me	*n*-Bu	H	H	158 AcOEt	83	$C_{12}H_{20}O_2N_4$	Calcd.	57.11	7.99	22.21
								Found	57.26	8.12	22.07
50	Me	Ph	H	H	168 AcOEt	91	$C_{14}H_{16}O_2N_4$	Calcd.	61.75	5.92	20.58
								Found	61.92	6.14	20.81
51	Me	H	Me	H	146 xylene	87	$C_9H_{14}O_2N_4$	Calcd.	51.42	6.71	26.65
								Found	51.37	6.79	26.76
52	Me	Me	Me	H	132—134 AcOEt	61	$C_{10}H_{16}O_2N_4$	Calcd.	53.55	7.19	24.99
								Found	53.47	7.00	25.05
53	Me	CH_2COOMe	Me	H	140 ligroin	63	$C_{12}H_{18}O_4N_4$	Calcd.	51.05	6.43	19.85
								Found	51.34	6.62	20.00
54	Me	Me	Me	Me	78—79 PE[a]	41	$C_{11}H_{18}O_2N_4$	Calcd.	55.44	7.61	23.52
								Found	55.42	7.63	23.19
55	Ph	Et	Me	H	107—108 ligroin	42	$C_{16}H_{20}O_2N_4$	Calcd.	63.98	6.71	18.65
								Found	64.08	6.78	18.89
56	Me	H	H	H	210—212 MeOH	82	$C_8H_{12}O_2N_4$	Calcd.	48.97	6.17	28.56
								Found	48.80	6.31	28.53
57	Me	H	Ph	H	203 EtOH	78	$C_{14}H_{16}O_2N_4$	Calcd.	61.75	5.92	20.58
								Found	61.78	6.25	20.76
58	Me	COOEt	Me	H	119—120 BuOH	85	$C_{12}H_{18}O_4N_4$	Calcd.	51.05	6.43	19.85
								Found	51.27	6.53	19.77
59	Ph	$CH_2NCH_2CH_2$ (Me)	H		196 AcOEt	81	$C_{17}H_{21}O_2N_5$	Calcd.	62.36	6.47	21.39
								Found	62.29	6.41	21.20

Ph=phenyl
a) PE: petroleum ether (bp 50—90°)

TABLE IV. 1,5,6,7-Tetrasubstituted 3-Methyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines



Compd. No.	R^1	R^5	R^6	R^7	mp (°C) Recryst. solvent	Yield (%) Method[a] (A)	(B)	Formula		Analysis (%) C	H	N
60	Me	H	Me	H	>300 EtOH	13(T)		$C_9H_{11}O_2N_3$	Calcd.	55.95	5.74	21.75
									Found	55.83	5.89	21.69
61	Me	Me	H	H	>300 EtOH	21(E)		$C_9H_{11}O_2N_3$	Calcd.	55.95	5.74	21.75
									Found	56.09	5.92	21.84
62	Me	Et	H	H	270—272 EtOH	30(E)		$C_{10}H_{13}O_2N_3$	Calcd.	57.96	6.32	20.28
									Found	57.68	6.55	20.36
63	Me	*n*-Bu	H	H	186—187 AcOEt	29(E)	41(T)	$C_{12}H_{17}O_2N_3$	Calcd.	61.25	7.28	17.86
									Found	61.49	7.35	17.83
64	Me	Me	Me	H	>300 EtOH	72(E)	72(E) 81(T)	$C_{10}H_{13}O_2N_3$	Calcd.	57.96	6.32	20.28
									Found	57.56	6.69	20.38

Compd. No.	R^1	R^5	R^6	R^7	mp (°C) Recryst. solvent	Yield (%) Method[a] (A)	(B)	Formula		Analysis (%) C	H	N
65	Me	Me	Me	Me	233—234 AcOEt		59(E)	$C_{11}H_{15}O_2N_3$	Calcd.	59.71	6.83	18.99
									Found	59.91	6.95	19.16
66	Me	Et	Me	H	287 AcOEt		68(E)	$C_{11}H_{15}O_2N_3$	Calcd.	59.71	6.83	18.99
									Found	59.70	6.95	19.02
67	Me	iso-Pr	Me	H	251—252 AcOEt		34(E)	$C_{12}H_{17}O_2N_3$	Calcd.	61.25	7.28	17.86
									Found	61.40	7.27	17.96
68	Me	*n*-Bu	Me	H	249 AcOEt		48(E)	$C_{13}H_{19}O_2N_3$	Calcd.	62.62	7.68	16.86
									Found	62.65	7.70	17.06
69	Me	iso-Bu	Me	H	271 AcOEt		62(E)	$C_{13}H_{19}O_2N_3$	Calcd.	62.62	7.68	16.86
									Found	62.79	7.82	16.98
70	Me	*n*-Am	Me	H	231 AcOEt		65(E)	$C_{14}H_{21}O_2N_3$	Calcd.	63.85	8.04	15.92
									Found	63.72	8.24	16.06
71	Me	Me	Et	H	268 EtOH–H_2O		72(E)	$C_{11}H_{15}O_2N_3$	Calcd.	59.71	6.83	18.99
									Found	59.86	6.97	19.02
72	Me	Et	*n*-Pr	H	217—218 AcOEt		76(E)	$C_{13}H_{19}O_2N_3$	Calcd.	62.62	7.68	16.86
									Found	62.76	7.76	17.04
73	Me	Me	Ph	H	285 EtOH		63(E)	$C_{15}H_{15}O_2N_3$	Calcd.	66.90	5.61	15.61
									Found	66.89	5.90	15.69
74	Me	Et	Ph	H	211 EtOH–H_2O		49(E)	$C_{15}H_{17}O_2N_3 \cdot H_2O$[b]	Calcd.	63.77	6.36	13.95
									Found	64.21	6.29	14.30
75	Me	*n*-Bu	Ph	H	200 EtOH–H_2O		42(E)	$C_{18}H_{21}O_2N_3$	Calcd.	69.43	6.80	13.50
									Found	69.39	6.90	13.47
76	Me	Ph	H	H	287 THF–ether	47(E)	54(T)	$C_{14}H_{13}O_2N_3$	Calcd.	65.87	5.13	16.46
									Found	65.81	5.34	16.31
77	Me	Ph	Ph	H	>300 EtOH		6(E)	$C_{20}H_{17}O_2N_3$	Calcd.	72.49	5.17	12.63
									Found	72.61	5.25	12.61
78	Me	$(CH_2)_3$		H	>300 EtOH–H_2O		78(E)	$C_{11}H_{13}O_2N_3$	Calcd.	60.26	5.98	19.15
									Found	60.18	5.76	19.33
79	Me	$(CH_2)_4$		H	>300 EtOH		86(E) 86(T)	$C_{12}H_{15}O_2N_3$	Calcd.	61.78	6.48	18.02
									Found	61.59	6.33	17.73
80	Ph	Me	Me	H	282 EtOH–H_2O		35(E) 95(T)	$C_{15}H_{15}O_2N_3$	Calcd.	66.90	5.61	15.61
									Found	66.64	5.53	65.67
81	Ph	Me	Me	Me	271 EtOH		65(T)	$C_{16}H_{17}O_2N_3$	Calcd.	67.82	6.05	14.83
									Found	67.86	6.08	14.70
82	Ph	Et	Me	H	218 EtOH–H_2O	43(T)	13(E)	$C_{16}H_{17}O_2N_3$	Calcd.	67.82	6.05	14.83
									Found	67.84	6.24	14.90
83	Ph	*n*-Bu	Me	H	214—215 EtOH–H_2O		42(E) 73(T)	$C_{18}H_{21}O_2N_3$	Calcd.	69.43	6.80	13.50
									Found	69.52	6.98	13.40
84	Ph	*n*-Bu	Me	Me	160 EtOH–H_2O		22(E)	$C_{19}H_{23}O_2N_3$	Calcd.	70.13	7.12	12.91
									Found	69.81	7.24	12.77
85	Ph	iso-Bu	Me	H	206 EtOH–H_2O		70(T)	$C_{18}H_{21}O_2N_3$	Calcd.	69.43	6.80	13.50
									Found	69.44	6.96	13.44
86	Ph	*n*-Am	Me	H	192 EtOH–H_2O		83(T)	$C_{19}H_{23}O_2N_3$	Calcd.	70.13	7.12	12.91
									Found	70.23	7.34	12.90
87	Ph	$(CH_2)_3$		H	>300 EtOH		94(T)	$C_{16}H_{15}O_2N_3$	Calcd.	68.31	5.38	14.94
									Found	67.73	5.42	14.93
88	Ph	$(CH_2)_4$		H	>300 EtOH		85(T)	$C_{17}H_{17}O_2N_3$	Calcd.	69.13	5.80	14.23
									Found	68.79	6.06	14.15
89	Me	CH_2-COOMe	Me	H	266 MeOH		53(T)	$C_{12}H_{15}O_4N_3$	Calcd.	54.33	5.70	15.84
									Found	54.41	5.95	16.10
90	Ph	CH_2-COOMe	Me	H	>300 MeOH		74(T)	$C_{17}H_{17}O_4N_3$	Calcd.	62.37	5.24	12.84
									Found	62.45	5.41	12.60
91	Me	CH_2-COOH	Me	H	284 H_2O		39	$C_{11}H_{13}O_4N_3$	Calcd.	52.58	5.22	16.73
									Found	52.31	5.33	16.59
92	Ph	CH_2-COOH	Me	H	242 EtOH–H_2O		92	$C_{16}H_{15}O_4N_3$	Calcd.	61.33	4.83	13.41
									Found	61.43	5.06	13.69

Ph=phenyl

a) reaction solvent: T, tetralin; E, ethylene glycol b) H_2O was confirmed by IR spectra.

Pharmacology

Diuretic activity (weight of urine for 3 hours in mice,[8] cardiac activity (chronotropic and inotropic),[9] CNS stimulating activity (spontaneous motor activity[10] and fighting activity[11]), and acute toxicity (LD_{50}) were tested concerning the resulting compounds. The results were given in Table V.

Table V. Diuretic, Cardiac, and CNS Stimulating Activities and Acute Toxicity of 7-Deazaxanthine Derivatives

Group	Compd. No.	Diuretic[a] activity		Cardiac[b] activities		CNS[c] stimulating activities		Acute toxicity[f] LD_{50} (mg/kg)
		100 mg/kg	200 mg/kg	Chronotropic	Inotropic	Spontaneous[d] motor activity	Fighting[e] activity	
A	6		2.4			++		168(119— 237)
	9	2.0	2.1	++	++	−	−	283(174— 461)
	14		1.2			−	−	476(338— 671)
	19		1.8					>800
	21		1.9				+	238(170— 336)
	23	1.3	2.7	++	++			200(134— 298)
	24		1.7				−	400(268— 596)
	26		1.4					>800
	64	2.3	3.2	+	+	++		400(245— 652)
	68	2.3	2.1			++	++	336(238— 474)
	73		1.5			+	++	>1600
	75		1.5	+	+		++	951(674—1341)
	80		1.1	−		−		1130
	83		1.2	−	−			>1600
	84		1.5					1130
	89		1.9					>1600
	90		0.7				+	>1600
	91		1.2				+	>1600
	92		0.8	+			++	>1600
B	30		2.6			+	++	336(238— 474)
	33	2.1	2.0	+	+	−		238(175— 231)
	40		1.5					>800
C	94		1.2					424(354— 508)
	95		0.9					692(633— 757)
	96		1.0					308(278— 343)
Caffeine		2.4	2.5	+	+	+++	+++	168(119— 237)
Theophylline		3.4	3.3	+		++	+++	238(190— 336)
Methamphetamine						++ (1 mg)	++ (5 mg)	15

a) Weight of urine for 3 hours in mice (*p.o.*) compared with control.[8] *b*) The effect on isolated guinea pig heart perfused by the Langendorff's method.[9] *c*) 50 mg/kg in mice (*i.p.*) *d*) postexploratory activity by the Animex apparatus (A.B. Farad Co.)[10] *e*) followed the method of Tedeschi[11] *f*) in mice (*i.p.*)

As to caffeine-like activities, **6**, **9**, **23**, **30**, **64**, and **68** showed the same or more diuretic activity than that of caffeine, **9** and **23** were more activity than caffeine in cardiac activity, and **68** and **73** showed the most CNS stimulating activity. Compounds (**64**—**92**) having substituents at 5- and 6-positions generally showed low toxicity. Pharmacological activities of A and B groups were nearly parallel. Namely, a double bond between 5- and 6-positions

8) T. Mineshita, S. Matsumura, S. Kimoto, and O. Uno, *Pharmacometrics*, 4, 33 (1970).
9) L. Ther, "Pharmakologische Methoden," Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1949, p. 170.
10) T.H. Svensson and G. Thieme, *Psychopharmacologia*, 14, 157 (1969); *idem*, *J. Pharm. Pharmacol.*, 22, 639 (1970).
11) R.E. Tedeschi, *J. Pharmacol. Exptl. Therap.*, 125, 28 (1959); G. Chen, *Arch. int. Pharmacodyn.*, 142, 30 (1963).

of compounds (A) had almost no effect on the pharmacological activities. In contrast to compounds of A and B groups, 2,4-dioxo-1,2,3,4,6,7-hexahydro-5H-cyclopenta[*d*]pyrimidines (C) did not show diuretic, cardiac, and CNS stimulating activities at all but weak analgetic activity like that of 1,3-disubstituted 5,6-dialkyluracils.[5,12] It indicates that the nitrogen atom at 7-position of compounds (A and B) plays an important role in showing caffeine-like pharmacological activities.

Experimental

1,3-Disubstituted 2,4-Dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines (II) (5—20 in Table I)——In 15 ml of H_2O were dissolved 8.2 g of AcONa and 39.3 g of 30% chloroacetaldehyde. The mixture was gradually added to a suspended solution (temperature 70—75°) consisting of 0.1 mole of 1,3-disubstituted 6-aminouracils (I)[8] and 8.2 g of AcONa in 50—100 ml of H_2O. The mixture was stirred for a few minutes at 80°, and cooled on standing. The resulting precipitate was filtered, washed with H_2O, and recrystallized.

1,3-Disubstituted 7-Methyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines (III) (21—27 in Table I)——1,3-Disubstituted 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidine (II: 6, 8, 9, 14, 17, 19, or 20) (0.01 mole) was dissolved in 10 ml of NaOH solution and the mixture was stirred for 1 hr with 1.5 g of Me_2SO_4 at 30—40°. The resulting crude crystals were filtered, washed with H_2O, and recrystallized.

1,3,7-Trisubstituted 2,4-Dioxo-1,2,3,4,5,6-hexahydropyrrolo[2,3-*d*]pyrimidines (IV) (28—42 in Table II)——A solution of 1,3,7-trisubstituted 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidine (III: 5—20, or 26) (0.01 mole) in EtOH (120 ml) was hydrogenated in an autoclave at 100—120° and 50—60 atm using Pd-C (0.5 g) as a catalyst. After 8—10 hr, the reaction solution was cooled to 80°, and activated carbon was added thereto. The catalyst was removed by filtration and washed well with EtOH. The filtrate and washings were collected and evaporated to dryness under reduced pressure. A small amount of acetone was added to the residue, the resulting crude crystals were filtered, washed with ether and recrystallized.

6-Hydrazino-3-methyl-1-phenyluracil (45)——To a solution of 6-chloro-3-methyl-1-phenyluracil[13] (30 g) in 50 ml of iso-PrOH was added 25 ml of 100% $NH_2NH_2 \cdot H_2O$, the mixture was refluxed for 30 min. After cooling, the precipitate was filtered, washed with H_2O, and recrystallized from MeOH to give 17.8 g of colorless crystals, mp 230—232°. *Anal.* Calcd. for $C_{11}H_{12}O_2N_4$: C, 56.89; H, 5.21; N, 24.13. Found: C, 57.06; H, 5.28; N, 24.39.

3-Methyl-6-(1-methylhydrazino)-1-phenyluracil (46)——To a solution of 6-chloro-3-methyl-1-phenyluracil (4.7 g, 0.02 mole) in 10 ml of iso-PrOH was added 5 ml of methylhydrazine and the mixture was refluxed for 5 min. Solvent was evaporated *in vacuo* and the residue was treated with H_2O. The precipitate was filtered, washed with H_2O, and recrystallized from AcOEt to give 3.2 g (62%) of colorless prisms, mp 146°. *Anal.* Calcd. for $C_{12}H_{14}O_2N_4$: C, 58.52; H, 5.73; N, 22.75. Found: C, 58.77; H, 5.88; N, 22.98.

1-Substituted 6-Alkylenehydrazino-3-methyluracils (VI) (47—59 in Table III)——a) To 50 ml of EtOH was added 0.01 mole of 6-hydrazino-1,3-dimethyluracil (43)[14a], then 0.012 mole of aldehydes (MeCHO, EtCHO, PrCHO, AmCHO, $PhCH_2CHO$) or ketones ($AcCH_2COOEt$, $AcCH_2CH_2COOEt$) were added, and the mixture was heated under reflux for 0.5—1 hr. After cooling, the resulting precipitate was filtered. Or the reaction solution was evaporated *in vacuo*, ether was added to the residue, and resulting crystals were collected. The crude products were recrystallized from a suitable solvent to give hydrazones (VI: 47—50, 53, 56, 58).

b) Reaction of ketones (acetone, MeCOEt, MeCOPr, MeCOPh, N-methyl-4-piperidone) and 6-hydrazinouracils (V: 43,[14a] 44,[14b] 45) were carried out in xylene in a similar manner as described above to give hydrazones (51, 52, 54, 55, 57, 59).

1,5,6,7-Tetrasubstituted 3-Methyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines (VII) (60—90 in Table IV)——Method A: A solution of 1-substituted 6-alkylenehydrazino-3-methyluracils (VI: 47—55) (0.01 mole) in 20 ml of tetraline (or ethylene glycol) was refluxed for 2—3 hr. After cooling, ether was added to the solution, the resulting precipitate was filtered to give crude products of 60—65, 76, 82, and 89.

Method B: To a solution of ketones (0.015 mole) in 20 ml of tetraline (or ethylene glycol) were added 0.01 mole of 6-hydrazinouracils (V: 43—46[14]) and refluxed for 2—3 hr. After cooling, ether (or water) was added to the reaction solution, the resulting precipitate was filtered, and recrystallized to give 64—90.

1-Substituted 5-Carboxymethyl-3,6-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines (91, 92 in Table IV)——1-Substituted 5-ethoxycarbonyl-3,6-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]-pyrimidine (89 or 90) (0.01 mole) was dissolved in 10—30 ml of 5% NaOH solution with heating on a water

12) S. Senda, M. Honda, K. Maeno, and H. Fujimura, *Chem. Pharm. Bull.* (Tokyo), 6, 490 (1958).
13) S. Senda, K. Hirota, and T. Asao, *Chem. Pharm. Bull.* (Tokyo), 22, 189 (1974).
14) *a*) W. Pfleiderer and K.H. Schundehutte, *Ann. Chem.*, 612, 158 (1958); *b*) H. Partenheimer, Ger. Patent 1186466 (1965) [*Chem. Abstr.*, 62, 13159 (1965)].

bath. After 5 min, the reaction solution was acidified with AcOH with cooling in ice water. The precipitate was filtered to give a crude product of 91 or 92.

Hydrolysis of 5-Ethoxycarbonyl-1,3,6-trimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidine (89) with Hydrochloric Acid——A solution of 1.8 g of 89 in 100 ml of 5% HCl was refluxed for 5 hr. After cooling, the resulting precipitate was filtered, washed with H_2O, and recrystallized from EtOH to give 1.0 g of 1,3,5,6-tetramethyl-2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidine (64). This compound was identified by comparison of infrared spectra with an authentic sample obtained in the preparation of VII.

1,3,5,6-Tetramethyl-2,4-dioxo-1,2,3,4,5,6-hexahydropyrrolo[2,3-*d*]pyrimidine (93)——64 (2.1 g, 0.01 mole) was hydrogenated in a similar manner as described in the preparation of IV. After the catalyst was removed by filtration of the hot reaction solution, on cooling to room temperature, 0.5 g of a starting material (64) was recovered by filtration. The filtrate was evaporated *in vacuo*, H_2O was added to the residue, the precipitate was filtered, and recrystallized from H_2O to give 0.3 g of 93; mp 237°. *Anal.* Calcd. for $C_{10}H_{15}O_2N_3$: C, 57.40; H, 7.23; N, 20.08. Found: C, 57.42; H, 7.40; N, 19.98.

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Safety Evaluation of 1,3-Dimethylamylamine (DMAA) in Dietary Supplement Products

Prepared for:

USPlabs LLC
Dallas, Texas

Prepared by:
Joseph V. Rodricks, Ph.D., DABT
Duncan Turnbull, Ph.D., DABT
Michael Lumpkin, Ph.D., DABT

ENVIRON International Corporation
Arlington, Virginia

Date:
May, 2012

FINAL REPORT

Introduction

ENVIRON International Corporation (ENVIRON) was asked to provide a safety assessment of 1,3-Dimethylamylamine (DMAA) as a dietary ingredient in the dietary supplements sold as Jack3d™ and OxyElite Pro™. ENVIRON has reviewed all of the available data relevant to the assessment of human health and safety and offers the following analysis and opinions.

In developing the safety assessment, ENVIRON reviewed and considered the following data, including data provided by USPlabs:

- A literature search performed by ENVIRON of the PubMed database and ToxNet search engine for published studies of DMAA (and chemical nomenclature synonyms), Jack3d, or OxyElite Pro,
- Six publications of human clinical studies of DMAA, Jack3d™ and/or OxyElite Pro™ in healthy men and women (Bloomer et al. 20011a, 2011b; Whitehead et al. 2012; McCarthy et al. 2012a, 2012b; Farney et al. 2012)
- Four studies in animals and humans published from 1927-1953 which inform on the pharmacological action of DMAA
- The U.S. patent for aminoalkanes (U.S. Patent Office 1944)
- A safety assessment of Jack3d™ performed by CANTOX Health Science International (CANTOX 2011a)
- A safety assessment of OxyElite Pro™ performed by CANTOX Health Science International (CANTOX 2011b),
- USFDA Adverse Event Reporting (AER) listing for dietary supplements (USFDA 2011).

The ToxNet search engine, maintained by the U.S. National Library of Medicine, is linked to the following databases: ChemIDplus, HSDB, Toxline, CCRIS, DART, GENETOX, IRIS, ITER, lact-Med, TRI, Multi-database, Hazmap, Household products, and TOXMAP.

Product Label Usage Direction and Warnings

The labels of both Jack3d™ and OxyElite Pro™ specify that use of these products should be limited to healthy adults, in consultation with a physician. The respective product labels also specify that Jack3d™ should be used no more than 5 days during any 7-day period, while OxyElite Pro™ should be used continuously for no more than 8 weeks, followed by a 4 week cessation of dosing. Both product labels state that no other source of caffeine should be used, nor should Jack3d™ or OxyElite Pro™ be combined with alcohol.

Jack3d™ is sold as a drink mix containing a proprietary blend of arginine α-ketoglutarate, β-alanine, creatine monohydrate, Schisandra chinensis extract, DMAA, caffeine, and excipient

fillers, flavorings, and colorants commonly found in foods. Per the label directions, 1 to 3 scoops (approximately 5.5 to 16.5 g) of Jack3d™ should be mixed with 4 to 8 fluid ounces (120 to 240 mL) of water and consumed approximately 45 minutes prior to physical exercise. Each scoop serving of 5.5 grams of Jack3d™ contains [redacted] DMAA and [redacted] mg of caffeine. Thus, the label-directed use of Jack3d™ on a daily basis would result in an exposure of [redacted] to [redacted] mg DMAA/kg/day and [redacted] to [redacted] mg caffeine/kg/day for a 70 kg individual.

OxyElite Pro™ is sold in capsules containing a proprietary blend of extracts of *Bauhinia purpurea L., Bacopa monnieri, Cirsium oligiphyllum*, and Yohimbe bark, as wells as caffeine, DMAA, and excipient fillers and colorants commonly found in other foods. Per the label directions, up to 3 capsules/day (2 capsules in the morning, followed by 1 capsule 8 hours later) may be taken. Each capsule contains [redacted] mg DMAA and [redacted] mg caffeine. Thus, the label-directed use of OxyElite Pro™ on a daily basis would result in an exposure of [redacted] to [redacted] mg DMAA/kg/day and [redacted] to [redacted] mg caffeine/kg/day for a 70 kg individual.

Characterization, Pharmacology, and Animal Toxicity of DMAA

DMAA is an aliphatic amine that, like other compounds in its class, acts in mammals as a sympathomimetic. That is, DMAA can mimic the effect, but not the intensity, of endogenously produced neuro-active catecholamines, such as epinephrine and norepinephrine (Figure 1), on the sympathetic nervous system. Effects include vasoconstriction, increase in blood pressure and heart rate, and bronchodilation.

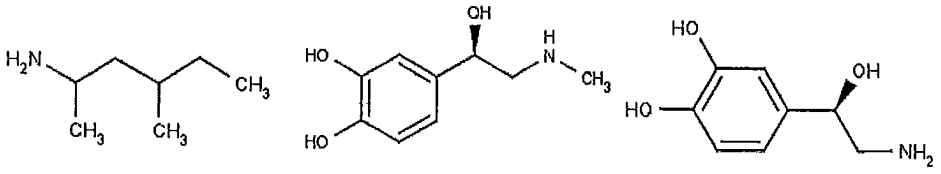

Figure 1. Chemical structures (left to right) of DMAA, epinephrine, and norepinephrine

Although there has existed some debate whether DMAA is a naturally occurring substance (Lisi et al. 2011), it has been definitively detected and quantified in extracts of the geranium plant, *Pelargonium graveloens* grown in the Guizhou Province of China (Ping et al. 1992; Li et al. 2012). The chemical structure, biological interactions, and pharmacological effects of naturally- or synthetically-derived DMAA are identical, making origin of DMAA irrelevant to the assessment of safety.

The ingredients in Jack3d™ and OxyElite Pro™ other than DMAA and caffeine are comprised of amino acid forms, various plant extracts, and creatine. Cantox (2011a, 2011b) reviewed the available laboratory animal data for the effects of these compounds, none of which are expected to contribute to responses of the sympathetic nervous system. This was confirmed in the clinical studies described below, as hemodynamic effects (heart rate and blood pressure), or lack thereof, were similar across the studies regardless of whether subjects ingested DMAA, caffeine, DMAA + caffeine, Jack3d™, or OxyElite Pro™.

DMAA was patented by Eli Lilly and Company in 1944 as a nasal decongestant and approved by U.S. FDA in1948. It was discontinued in 1978, and the NDA (6444) was withdrawn by Eli Lilly in 1983. It was sold as an over-the-counter (OTC) medication because of its ability – similar to ephedrine and amphetamine – to induce vasoconstriction in and reduce swelling of nasal tissues, but without the potent central nervous system stimulation induced by those two compounds (U.S. Patent Office, 1944). From 1948-1978, DMAA was marketed as Forthane® and provided in a nasal inhaler (the M-52 inhaler), which contained 250 mg DMAA. DMAA was reported to induce vasopressor activity of about $1/200^{th}$, $1/7^{th}$, and $1/225^{th}$ that of epinephrine in cats, rats, and dogs, respectively (Rohrman and Shonle 1927; Swanson and Chen 1946; Miya and Edwards 1953). Lethal doses in 50% of test animals (LD_{50}) are 185 mg/kg ip in mice and 39-73 mg/kg iv in mice and rats, respectively (Swanson and Chen 1946; Marsh et al, 1951). However, there are no data for lethality following oral exposures, which would be expected to exceed lethal doses given by intravenous or intraperitoneal injection.

Pharmacological Similarities Between DMAA and Caffeine

DMAA and caffeine have similar effects on hemodynamic properties (i.e., heart rate and blood pressure), but differ in effective dose. Single 250 mg caffeine doses (the approximate caffeine dose in 2-3 cups of coffee) or 50 mg DMAA doses in healthy adults resulted statistically similar transient increases in systolic (6-16 mm Hg) and diastolic (6-9 mm Hg) blood pressure (Bloomer et al 2011a; Robertson et al. 1978; Nurminen et al. 1999). These blood pressure changes are offset by reduced heart rate (4-5 bpm) to maintain consistent cardiovascular load (Bloomer et al. 2011b).

Available Human Clinical Data Relevant to Safety Assessment

Six human clinical studies reporting health effects from DMAA and caffeine, Jack3d™, and OxyElite Pro™ use have been performed by researchers at the University of Memphis, published in the peer-reviewed scientific literature, and are summarized below as well as in Table 1. These studies report exposure durations ranging from single doses to 2, 8, and 10 weeks. The effects reported in these studies published in the last two years comport with effects reported for in the older literature, where oral ingestion of 3 mg/kg DMAA in adult male volunteers (over 3-fold

higher than the maximum labeled dose for Jack3d™ or OxyElite Pro™) resulted in a transient increase in systolic blood pressure beginning at about 30 minutes and decreasing after 100 minutes (Marsh et al. 1951).

Bloomer et al. (2011a) investigated the addition of DMAA and caffeine on resting hemodynamic properties and endogenous sympathetic catecholamine (epinephrine and norepinephrine) levels of volunteers for up to 2 hours after dosing. Five male and five female healthy adults consumed a single dosing of 250 mg caffeine (C) (2.8-3.4 mg caffeine/kg), 50 mg DMAA (D50) (0.6-0.7 mg DMAA/kg), 75 mg DMAA (D75) (0.9-1.0 mg DMAA/kg), or combinations of 250 mg caffeine plus 50 (C+D50) or 75 (C+D75) mg DMAA. Heart rate, diastolic blood pressure, and plasma levels of epinephrine or norepinephrine were not significantly different across all treatment groups or from pre-ingestion (control) values. After 60 minutes, systolic blood pressure was increased in all treated groups (122-143 mm Hg in a dose-related manner) above pre-ingestion values (117-121 mm Hg), with D75 and C+D75 producing higher values (132 and 141 mm hg, respectively) than C alone (122 mm Hg) at 90-120 minutes. Likewise, diastolic pressure in all treated groups (76-83 mm Hg) was higher than pre-ingestion values (68-71 mm Hg) after 60 minutes, but combining DMAA with caffeine resulted in values similar to caffeine alone. The rate pressure product (heart rate × systolic blood pressure) increased with DMAA dose. Epinephrine and norepinephrine levels in plasma did not increase in treated groups, suggesting that the reported changes in blood pressure by caffeine and/or DMAA are not mediated by induction of catecholamines, but possibly by direct stimulation of sympathetic receptors.

Bloomer et al. (2011b) administered caffeine, DMAA, or combinations of both to volunteers prior to them running 10 km. Six males and 6 females with an average age of 22 years were given 0, 4 mg caffeine/kg, 1 mg DMAA/kg, or a combination of 4 mg caffeine/kg and 1 mg DMAA/kg, in 500 ml water. Treatments were ingested one hour prior to running 10 km on an outdoor track. Each subject completed four test runs with a different treatment before each run, with one week in between each test. Air temperatures during each test ranged from 44°F to 68°F. There were no statistically significant differences between groups in required run time, perceived exertion, self-reporting of mood and vigor, and heart rate during the run. At 5 and 30 minutes post-exercise, the heart rate in the caffeine+DMAA group was higher than the caffeine or DMAA groups, but not the placebo group. Systolic blood pressure in the caffeine+DMAA group at 5 and 30 minutes post-exercise (126 mm Hg) was similar to placebo (126 mm Hg), but lower than the caffeine-alone (141 mm Hg) or DMAA-alone (147 mm Hg) groups. Diastolic blood pressure at 5 minutes post-exercise was similar across groups (64-66 mm Hg), but lower in the DMAA+caffeine group (61 mm Hg). The rate pressure product was similar in the placebo and DMAA+caffeine groups at 5 and 30 minutes post-exercise, but higher in the caffeine-alone or DMAA-alone groups. These data indicate that a combination of 1 mg DMAA/kg and 4 mg caffeine/kg, a dose level approximately equivalent to the maximum product label dose, did not

significantly change physical performance, level of exertion, subject mood or vigor, heart rate, or blood pressure endpoints, compared to placebo, following a very strenuous physical activity.

McCarthy et al. (2012a) examined the effect of single doses of OxyElite Pro™ on hemodynamics of healthy adults for up to two hours after treatment. Six males and 6 females were administered two capsules of OxyElite Pro™ ([redacted] mg DMAA/kg and [redacted] mg caffeine/kg) or placebo on two separate days in a cross-over study design. An increase in heart rate of 8-11 beats/min (BPM) was reported in the treated group beginning at 60 minutes. Systolic blood pressure increased (112-118 mm Hg) in the treated groups, compared to placebo (101-104 mm Hg) beginning at 30 minutes after dosing. The rate pressure product increased in the treated group at 60 minutes after dosing. There was no increase in diastolic pressure.

Farney et al. (2012) investigated hemodynamic, hematological, and clinical chemistry effects of Jack3d™ after single and 14-day dosing. Seven healthy adult males consumed two scoops (11 g) of Jack3d™ in water for 14 days, resulting in DMAA and caffeine doses of [redacted] and [redacted] mg/kg/day, respectively. After dosing on days 1 and 14 (acute-phase observations), systolic blood pressure increased (122-123 mm Hg) over pre-ingestion values (109 mm Hg) beginning at 30 minutes. There were no significant differences in acute changes in heart rate, diastolic pressure, or rate pressure product on days 1 or 14. After 14 days of dosing, no significant changes in hemodynamic endpoints compared to day 1 were reported. Further, 14 days of dosing did not affect results of blood tests, including complete blood counts and lipid and metabolic panels.

Farney et al. (2012) also investigated hemodynamic, hematological, and clinical chemistry effects of OxyElite Pro™ after single and 14-day dosing. Four healthy adult males and two females consumed two capsules of OxyElite Pro™ for 14 days, providing DMAA and caffeine doses of [redacted] and [redacted] mg/kg, respectively. After dosing on days 1 (acute-phase observations), systolic blood pressure increased (116-119 mm Hg) over pre-ingestion values (103 mm Hg) beginning at 60 minutes. There were no significant differences in acute changes in systolic pressure on day 14, or in heart rate, diastolic pressure, or rate pressure product on days 1 or 14. After 14 days of dosing, no significant changes in hemodynamic endpoints compared to day 1 were reported. Further, 14 days of dosing did not affect results of blood tests, including complete blood counts and lipid and metabolic panels.

McCarthy et al. (2012b) examined the effect of an 8-week exposure of OxyElite Pro™ on hemodynamic, hematological, and clinical chemistry endpoints. Groups of 16 healthy, adult males and females consumed 1-2 capsules OxyElite Pro™ or two placebo capsules daily for 8 weeks, resulting in daily DMAA and caffeine doses of [redacted] and [redacted] mg/kg, respectively. In the treated group, resting heart rate was slightly, but statistically significantly, higher (69.4 BPM) at the end of the study compared to the beginning (63.3 BPM), but were not different from the placebo control values (65-67 BPM). There were no differences between treatment groups or

pre- or post-study values for systolic or diastolic blood pressure. There were no clinically-relevant differences between treatment groups or across time in hematology, lipid, or metabolic panel endpoints.

Whitehead et al. (2012) examined the effect of a 10-week exposure of Jack3d™ on hemodynamic, hematological, and clinical chemistry endpoints. Groups of 12 or 13 healthy, adult males consumed 1-3 scoops (5.5-16.5 g) of Jack3d™ or placebo powder in water prior to exercise on an average of 4 days/week for 10 weeks. This treatment regimen resulted in DMAA and caffeine exposure ranges of [redacted] and [redacted] mg/kg and exercise days. Ten weeks of Jack3d™ use resulted in reported heart rate and systolic and diastolic blood pressure values similar to placebo controls. There were no clinically-relevant differences between treatment groups or across time in hematology, lipid, or metabolic panel endpoints.

There are two additional studies currently being conducted by Bloomer et al. The clinical phase of a pharmacokinetic study to profile DMAA concentration in the blood of 8 male adults consuming 50 mg of DMAA in capsules has been completed. The study investigators also measured heart rate, blood pressure, and body temperature over a 12-hour period post-dosing and again at 24 hours. A 12-week placebo-controlled dietary intervention study is being performed in which groups of 15 adult men will consume placebo, 50 mg DMAA, 250 mg caffeine, or 50 mg DMAA + 250 mg caffeine daily in capsules. Endpoints to be measured at 0, 6, and 12 weeks include hemodynamic parameters, clinical chemistry, hematology, urinalysis, blood markers for oxidative stress, inflammation, and cardiac muscle damage, and electrocardiography. Every two weeks, participants will also self-report endpoints including mild, moderate, or severe changes noticed in heart rate, sleep quality, mental focus, and physical performance. This study is currently scheduled to be completed in 2012.

The clinical studies for DMAA, Jack3d™, and OxyElite Pro™ contain similar findings for the effect of DMAA administered orally with or without caffeine: a transient increase in systolic blood pressure of approximately 12-18% occurs approximately 60-90 minutes after ingestion (Table 1). This is expected, given the sympathomimetic nature of DMAA. Extended exposure exposures of 2 to 10 weeks, either daily or on workout days only, did not result in exposure duration-related increases in resting heart rate or blood pressure. A 12-18% increase in systolic pressure, 10-15% increase in diastolic pressure (in one study, Bloomer et al. 2011a), and 6% increase in heart rate (as seen in one study, McCarthy et al. 2012) in healthy adults for periods of 1-2 hours per day does not constitute an adverse health effect and would not be expected to have long-term adverse consequences on cardiac health. This is particularly true if Jack3d™ or OxyElite Pro™ is used just prior to workouts, as strenuous exercise results in a transient increase in systolic pressure. In fact, no increase in blood pressure was reported after a 10 km run by runners who consumed DMAA+caffeine beforehand (Bloomer et al. 2011b). The similarity for hemodynamic results for DMAA alone or as a component in Jack3d™ or OxyElite Pro™

indicate that the other product components (i.e., amino acid forms, creatinine, and plant extracts) did not influence these endpoints.

The results of the hematological, metabolic, and lipid panel tests in the 2- to 10-week studies indicate that Jack3d™ and OxyElite Pro™ use over an extended period of time does not adversely impact liver or kidney function, as indicated by clinically normal plasma levels of bilirubin, alkaline phosphatase, and aspartate and alanine transaminases, and gamma glutamyl transferase (liver), as well as glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, and albumin (kidney). Self-reporting of no incidents of discomfort or elevated body temperature during an extended strenuous physical activity (10 km run) or after 10 weeks of episodic workouts indicate that labeled uses of OxyElite Pro™ or Jack3d™ does not increase the susceptibility to induction of hyperthermia or syncope in healthy adults.

Analysis of Association of Adverse Heat-Related Health Effects with Consumption of Jack3d™ and OxyElite Pro™

DMAA-containing supplements such as Jack3d™ and OxyElite Pro™ may be used during extreme heat conditions, which can be associated with effects such as loss of consciousness, hyperthermia, muscle breakdown during exertion, and rapid heartbeat, and kidney and liver failure. An important issue to resolve is whether exposure to dietary supplements containing DMAA and caffeine imparts significant additional risk of causing these or other effects under conditions of extreme heat and physical exertion. The clinical data for DMAA + caffeine, Jack3d™, and OxyElite Pro™ indicate that clinical precursors leading to each of the adverse effects of concern have not been observed, as shown in the following discussion.

Loss of consciousness (syncope): None of the subjects ingesting DMAA at Jack3d™ or OxyElite Pro™ labeled doses reported light-headedness or loss of consciousness during or after a 10 km run (Bloomer et al. 2011b) or while using either product for up to 10 weeks in conjunction with a frequent exercise workout regimen (Farney et al. 2012; McCarthy et al. 2012b; Whitehead et al. 2012). Hemodynamic data from all 6 clinical studies of DMAA never indicate conditions of blood pressure drop that could be associated with diminution of conscious faculties.

Heat injury (hyperthermia): A chemically-induced increase in risk of exertional hyperthermia requires interference with the ability of the body to shed excess heat. This interference may be caused by dehydration, significant decrease in electrolyte concentrations, and/or inhibition of sweat gland function leading to loss of evaporative cooling at the skin surface (Armstrong et al. 2007a). Human studies have shown that caffeine ingestion of less than 600 mg/day in adults does not result in increased diuresis (fluid loss to urine) (Armstrong et al. 2005, 2007b). No data were available to demonstrate the effect of DMAA or other aliphatic amines on diuresis, but epinephrine and norepinephrine (more potent sympathomimetics than DMAA) do not increase diuresis (Billewicz-Stankiewicz et al. 1980). Healthy adults administered single exposures of

DMAA+caffeine or Oxy Elite Pro either resting (Bloomer et al. 2011a; McCarthy et al. 2012 a, 2012b) or prior to running 10 km (Bloomer et al. 2011b) did not report in increase in subjective indicators of thirst, uncharacteristically perfuse sweating, or urinary urge. In a study of healthy adults using DMAA prior to running 10 km in ambient air temperatures ranging from 44°F to 68°F, there was no indication from study subjects of thermal discomfort or change in required exertion level, compared to an identical run performed by the same subjects after consuming a placebo (Bloomer et al. 2011b). These findings from subjects performing very strenuous physical exercise at relatively mild ambient temperatures are useful in that significant changes to body heat regulation would be detected and reported without confounding by high ambient air temperatures. Similarly, subjects using Jack3d™ or OxyElite Pro™ at labeled doses prior to exercise workout for 2 to 10 weeks did not report thermal discomfort (Farney et al. 2012; McCarthy et al. 2012b; Whitehead et al. 2012). Blood clinical chemistry results from the same subjects did not indicate any effect on electrolyte concentrations that could be magnified if exercising in conditions of extreme heat. Thus, clinical data indicate that labeled use of Jack3d™ or OxyElite Pro™ would not increase the risk of heat injury for a healthy adult performing strenuous physical activity in hot conditions.

Exertion-induced muscle breakdown (rhabdomyolysis): Clinical manifestation of exertional rhabdomyolysis may include muscle pain, swelling, and weakness, electrolyte imbalance, decreased renal function, abnormal heart rate, confusion, and gastrointestinal distress. None of these signs, symptoms, or indications from clinical chemistry results from users of labeled doses of Jack3d™ or OxyElite Pro™ were reported in the six clinical studies of DMAA. Thus, use of Jack3d™ or OxyElite Pro™ at labeled doses is unlikely to increase the risk for developing acute or chronic exertional rhabdomyolysis in healthy adults.

Rapid heartbeat (tachycardia): Heart rate data from the six clinical studies of DMAA do not indicate the occurrence of rapid heartbeat/tachycardia, even in subjects using DMAA and caffeine prior to a 10 km run.

Liver failure: Blood samples subjected to metabolic panel examinations were reported for subjects using Jack3d™ or OxyElite Pro™ for 2 to 10 weeks (McCarthy et al, 2012b; Farney et al. 2012; Whitehead et al. 2012). Indications of liver health included blood levels of bilirubin, alkaline phosphatase, aspartate and alanine transaminases, and gamma glutamyl transferase. In all of the multi-dose studies, these parameters were all well within clinical reference ranges, indicating the lack of evidence for subclinical precursors to liver injury or failure.

Kidney failure: Blood samples subjected to metabolic panel examinations were reported for subjects using Jack3d™ or OxyElite Pro™ for 2 to 10 weeks (McCarthy et al, 2012b; Farney et al. 2012; Whitehead et al. 2012). Indications of kidney health included blood levels of glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, and albumin. In all of the multi-dose studies, these parameters were all well within clinical reference ranges. Normal electrolyte and

plasma protein levels provided no indications of onset of metabolic acidosis that could accompany kidney failure. Thus, there was no indication of evidence for subclinical precursors to kidney injury or failure.

The USFDA maintains a database of user- and clinician-reported adverse events occurring simultaneously with use of dietary supplements as a means of public health surveillance in the U.S. (USFDA 2012). The Adverse Event Reporting (AER) database is updated monthly and analyzed by FDA staff to detect evidence for association of adverse events with use of specific supplements. As evidence points to such associations, USFDA issues AER alerts to the public. To date, no AER alerts have been issues for Jack3d™, OxyElite Pro™, or other DMAA-containing dietary supplements.

There are no data available for effects arising from co-exposures of Jack3d™ or OxyElite Pro™ and other dietary supplements. Authors of a case report of cerebral hemorrhage in a 21-year-old male implicated a mixture of abusive bolus doses of DMAA (approximately 600 mg), caffeine (150 mg), and alcohol as possibly causative (Gee et al. 2010). Such co-exposures are explicitly contraindicated on the Jack3d™ and OxyElite Pro™ product labels. Furthermore, the dose of DMAA consumed in this case was ten-times higher than the maximum recommended daily intake from Jack3d™ or OxyElite Pro™.

Conclusions

The use of DMAA in humans has been documented since the 1940s. It is a naturally occurring aliphatic amine that has sympathomimetic properties, was marketed as an FDA-approved OTC nasal decongestant for 30 years, and is used as a stimulant in dietary supplements, such as Jack3d™ and OxyElite Pro™. Over the past two years, six published clinical studies of DMAA, Jack3d™, and OxyElite Pro™ provided data on the hemodynamic, hematological, liver, and renal safety of these products in healthy adults consuming labeled doses for up to 10 weeks (McCarthy et al, 2012a, 2012b; Farney et al. 2012; Bloomer et al. 2011a, 2011b; Whitehead et al. 2012). This same group of investigators is currently performing two additional clinical studies to inform on the pharmacokinetics of DMAA and to add additional clinical observations throughout 12-weeks of DMAA use by larger groups of volunteers than previously tested. The stimulatory hemodynamic effects, including short-term increases in blood pressure, of DMAA in Jack3d™ and OxyElite Pro™ at labeled usage rates are statistically identical to those from the amount of caffeine in 2-3 cups of coffee. The clinical data indicate lack of changes in clinical markers that would be exhibited as precursors or manifestations of clinically adverse outcomes. Thus, there is no scientific evidence that labeled use of these products by healthy adults will compromise individual health or increase susceptibility to heat-related injuries.

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Table 1. Summary of Effects from Six Published Clinical Studies of DMAA, Jack3d™, or OxyElite Pro™

			Effects Observed / Reported					
Study	Number of Subjects	Exposure Intensity and Duration	Heart Rate	Systolic Blood Pressure	Diastolic Blood Pressure	Rate Pressure Product	Hematology	Metabolic Panel
Bloomer et al. (2011a)	5 males 5 females	Single exposure of DMAA (0.6-0.7 or 0.9-1.0 mg/kg) and/or caffeine (2.8-3.4 mg/kg)	NCRC	↑6-18%, decreasing after 90 min	↑10-15%	↑9%	NT	NT
Bloomer et al. (2011b)	6 males 6 females	Single exposure of placebo, DMAA (1 mg/kg), caffeine (4 mg/kg), or both	NCRC	↑17%, decreasing to baseline after 30 min post-exercise	NCRC	NCRC	NT	NT
McCarthy et al (2012a)	6 males 6 females	Single exposure (2 capsules) Of Oxy Elite Pro ([redacted]mg DMAA/kg And [redacted] mg caffeine/kg)	↑ 6% thru 2 hrs	↑15% thru 2 hrs	NCRC	↑22%	NT	NT
Farney et al. (2012a)	7 males	Single exposure (2 scoops) of Jack3d ([redacted] mg DMAA/kg and [redacted] mg caffeine/kg)	NCRC	↑12%, decreasing after 60 min	NCRC	NCRC	NT	NT
Farney et al. (2012a)	4 males 2 females	Single exposure (2 capsules) of Oxy Elite Pro (0.6 mg DMAA/kg and 3 mg caffeine/kg)	NCRC	↑16%, decreasing after 90 min	NCRC	NCRC	NT	NT
Farney et al. (2012a)	7 males	14-day exposure (2 scoops/day) of Jack3d ([redacted] mg DMAA/kg and [redacted] mg caffeine/kg)	NCRC	↑13%, decreasing after 90 min	NCRC	NCRC	NCRC	NCRC
Farney et al. (2012a)	4 males 2 females	14-day exposure (2 capsules/day) of Jack3d ([redacted] mg DMAA/kg and [redacted] mg caffeine/kg)	NCRC	↑14%, decreasing after 60 min	NCRC	NCRC	NCRC	NCRC
McCarthy et al (2012b)	16 males 16 females	8-week exposure (2 capsules/day) of placebo or Oxy Elite Pro ([redacted] mg DMAA/kg and [redacted] mg caffeine/kg)	NCRC	NCRC	NCRC	NCRC	NCRC	NCRC
Whitehead et al. (2012)	30 males	10-week exposure (2.4 scoops/day) of placebo or Jack3d ([redacted] mg DMAA/kg and [redacted] mg caffeine/kg)	NCRC	NCRC	NCRC	NCRC	NCRC	NCRC

NCRC = No clinically-relevant changes; NT = Not Tested

A Comparison of the Physiological Effects of Caffeine and Dimethylamylamine (DMAA)

The compound, 1,3-dimethylamylamine (DMAA) is a central nervous system stimulant added to some dietary supplements. It shares similar pharmacological effects in humans with caffeine (1,3,7-trimethylxanthine), a central nervous system stimulant consumed worldwide in the diet.

Both DMAA and caffeine are amine, or alkaloid-type, compounds occurring naturally in plants. Caffeine is found in coffee, tea, cacao beans (source for chocolate and cocoa) guarana, mate, bissy nuts and kola nuts, though the compound has been identified in more than 60 plant species (Frary et al. 2005, Barone et al. 1996). DMAA has been found in parts-per-billion to parts-per-million concentration levels in both geranium (*Pelargonium graveolens*) plant tissue (stems and leaves) and distilled plant oil (Li et al. 2012, Ping et al. 1992, USPlabs 2012).

Caffeine is consumed by more than 80% of the world's population each day and 82-87% of the U.S. population (Frary et al. 2005, Heckman et al. 2012). Published values of average daily caffeine intake from beverage consumption in the U.S. range from 106-170 mg/day for adults and 120 mg/day for all ages (Knight et al. 2004). Total average daily caffeine intake in the U.S. from food and beverages is 227-300 mg/day for adults and 193 mg/day for all consumers (Frary et al. 2005, Knight et al. 2004). Caffeine consumption by U.S. adults, expressed on a per body weight basis, was reported to be approximately 4 mg/kg/day (Knight et al. 2004), which can be attained by consuming 2-4 cups of coffee or 2-6 cups of brewed tea. Outside of the U.S., daily average caffeine intake of 400 mg/day (or 6 mg/kg/day for a 70 kg adult) has been reported (Biaggioni and Davis 2002), with average intake in Denmark reported to be 7 mg/kg/day (Barone et al. 1996).

Nawrot et al. (2003), in their comprehensive review of the literature, estimated a safe level of daily caffeine consumption of 400 mg/day, which was not associated with adverse health effects for healthy adults. However, doses as high as 750 mg/day have also been shown to be well tolerated in normal subjects (Biaggioni and Davis 2002), while patients with cardiovascular disease exhibited favorable tolerance for doses of up to a 250 mg dose (Hirsch et al. 1989).

The similarities between caffeine and DMAA for physiological changes in hemodynamic effects were reported in adults in a randomized, double-blinded, crossover clinical study (Bloomer et al. 2011). Ten healthy men and women were given 250 mg caffeine or 50 mg DMAA while at a rest. Caffeine ingestion resulted in an average maximum increase in systolic (SBP) and diastolic blood pressure (DBP) of 6 mm Hg and a decrease in heart rate of 5 beats per minute (bpm) over a 120 minute period after administration. A 50 mg dose DMAA resulted in an average maximum increase in SBP and DBP of 7 and 8 mm Hg, respectively, while heart rate decreased by 4 bpm. The changes in blood pressure and heart rate following doses of 250 mg caffeine or 50 mg DMAA were not statistically different.

In this same study, Bloomer et al. (2011a) reported that doses of 75 mg DMAA (which is 25% to 275% greater than in a labeled single serving of the USPlabs products OxyElite Pro™ or Jack3d™) resulted in average maximum increases in SBP and DBP of 16 and 9 mm Hg, respectively, along with a decrease in heart rate of 3 bpm. The increases in SBP and DBP from 75 mg DMAA doses are not significantly different from those reported in other clinical studies involving similar subject populations. Robertson et al. (1978), in a double-blind crossover clinical study, gave 250 mg caffeine to nine young, healthy men and women at rest. The average maximum increase in SBP and DBP was 14 mm Hg and 10 mm Hg, respectively, while heart rate decreased initially and then increased slightly. Nurminen et al. (1999) reported that a 250 mg caffeine dose in adults produced an average maximum increase in SBP and DBP of 12 mm Hg and 13 mm Hg, respectively. In a single-dose study evaluating the hemodynamic effects of Jack3d™, Farney et al. (2012) reported that a double serving of the product, providing 40 mg of DMAA and 250 mg of caffeine, resulted in an average maximum increase in SBP and DBP of 13 and 8 mm Hg. DMAA and caffeine also share similar hemodynamic effect profiles over time; peak magnitude of effects appear within 30-60 minutes post-administration, followed by a gradual decline to baseline (Hirsch et al. 1989, Robertson et al. 1978, Farney et al. 2012, Marsh et al. 1951, Mort and Kruse 2008). Thus, the effects of DMAA consumed in labeled servings of Jack3d™ and OxyElite Pro™ upon blood pressure are quite similar to those seen with a 250 mg dose of caffeine, the amount found in 2-3 cups of coffee or 2-6 cups of brewed tea.

For caffeine, the transient reduction in heart rate concomitant with the increase in blood pressure is thought to arise from the baroreceptor reflex, in which an increase in blood pressure results in

a decrease in heart rate (Lane and Manus 1989). This homeostatic mechanism aids in maintaining a steady total cardiac workload. It is only when the baroreceptor reflex is overcome that this does not occur. The initiation of the baroreceptor reflex was indicated in clinical studies of resting adults who consumed DMAA (Bloomer et al. 2011a, Farney et al. 2012, Whitehead et al. 2012, Marsh et al. 1951). Additionally, strenuously exercising adults who consumed DMAA or placebo exhibited no difference in heart rate, indicating that labeled DMAA use does not increase cardiac workload (Bloomer et al. 2011b). Tachyphylaxis, the partial tolerance to changes in blood pressure after an initial administration, appears to occur for both caffeine and DMAA (25, 26).

Both caffeine and DMAA have both exhibited very good tolerance by adults. Caffeine consumption levels substantially higher (400-500 mg/day) than those typically (275-300 mg/day) consumed are considered well tolerated (Kivity et al. 1990). Similarly, DMAA was shown to be well tolerated alone or in finished dietary supplement formulations (Bloomer et al. 2011a, Farney et al. 2012, Bloomer 2012, Whitehead et al. 2012, Bloomer et al. 2011b, McCarthy et al 2012a, McCarthy et al. 2012b) with no adverse health impacts reported. Furthermore, a dose 3 mg/kg DMAA dose (3.5 to 10.5 times greater than DMAA in labeled servings of OxyElite Pro or Jack3d for a 70 kg adult) has also been explored in a small group of individuals and also was shown to be well tolerated, with no serious adverse events. Recent clinical trials of DMAA and caffeine consumed in combination provide hemodynamic data for subjects that ingested both compounds just prior to exercise (Farney et al. 2012, Whitehead et al. 2012, Bloomer et al. 2011b), post exercise (Bloomer et al. 2011b), and over the course of time in which regular exercise was performed (Farney et al. 2012, Whitehead et al. 2012, McCarthy et al. 2012b). The available data for DMAA and caffeine do not indicate that consumption of both compounds in the dietary supplements OxyElite Pro™ or Jack3d™ would increase the susceptibility of adults to adverse cardiovascular events while exercising.

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