Short communication

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A synthetic stimulant never tested in humans, 1,3-dimethylbutylamine (DMBA), is identified in multiple dietary supplements

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A synthetic stimulant never before studied in humans, 1,3-dimethylbutylamine (DMBA), was suspected of being present in dietary supplements. DMBA is an analogue of the pharmaceutical stimulant, 1,3-dimethylamylamine (DMAA), which was recently banned by the US Food and Drug Administration. We obtained all dietary supplements sold by US distributors that listed an ingredient on the label, such as AMP Citrate, that might be a marketing name for DMBA. Supplements were analyzed for the presence and quantity of DMBA. Fourteen supplements met our inclusion criteria and were analyzed by two separate laboratories using ultra high performance liquid chromatography (UHPLC) - mass spectrometry and a reference standard. The identity of DMBA was confirmed in 12 supplements in the range of 13 to 120 mg DMBA per serving. Following recommendations on the supplement label for maximum daily intake, customers would consume from 26 to 320 mg of DMBA per day. Supplements containing DMBA were marketed to improve athletic performance, increase weight loss and enhance brain function. DMBA has never before been detected in supplements. The stimulant has never been studied in humans; its efficacy and safety are entirely unknown. Regulatory agencies should act expeditiously to warn consumers and remove DMBA from all dietary supplements. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: dietary supplements; adulteration; stimulants

Introduction

In the United States (US), the law governing dietary supplements permits supplements to be marketed to improve weight loss, enhance athletic performance as well as a wide range of other indications.[1] No evidence of efficacy or safety in humans is required.[2] This regulatory framework creates the perverse incentive for unethical manufacturers to place medications and other active pharmaceutical ingredients in supplements to boost sales.

When adulterated with active pharmaceutical ingredients, dietary supplements pose serious health risks to consumers.[3–7] Compounding the risks, the US Food and Drug Administration (FDA) and other regulatory agencies have had significant difficulty identifying and removing adulterated supplements in a timely fashion.[6] As these products remain readily available to the public, consumers continue to be exposed to unacceptable risks.

The stimulant 1,3-dimethylamylamine (DMAA) exemplifies the risks of supplements containing pharmaceutical drugs. A pressor amine introduced by Eli Lilly in 1948 as a nasal decongestant, DMAA was withdrawn from the US market over 40 years ago.[7–9] Reintroduced in 2006 as a dietary supplement ingredient, DMAA was sold in dozens of sports and weight loss supplements with over $100 million (USD) in sales in 2010 alone.[7] However, concerns about DMAA's health risks have led regulatory agencies in the USA, the UK, the Netherlands, Brazil, and elsewhere to ban or otherwise pressure manufacturers to remove DMAA from supplements. DMAA continues to be investigated as a cause of strokes, heart failure, and sudden death.[10]

While regulatory authorities have focused on removing DMAA from supplements, new synthetic stimulants have appeared in presumably 'natural' supplements. For example, just in the past year, two stimulants – β-methyl-phenylethylamine (BMPEA) and N,α-diethylphenylethylamine (DEPEA) – were discovered in dietary supplements in the USA, Europe and elsewhere.[11,12]

In the present study, supplements were analyzed for the presence and quantity of an analogue of DMAA, 1,3-dimethylbutylamine (DMBA) also known as 2-amino-4-methylpentane and 4-methyl-2-pentanamine (Figure 1). DMBA has not previously been described in dietary supplements, but we hypothesized that AMP Citrate, 4-amino-2-methylpentane citrate, and several other marketing names listed on supplement labels might refer to DMBA.

Our objective was to obtain all dietary supplements available for sale by US distributors listing ingredients that we suspected might refer to DMBA. Inclusion criteria were: (1) dietary supplements available for purchase from a US distributor in April 2014; and (2) supplements labelled as containing one or more of the following synonyms that might be used by supplement manufacturers for DMBA: 4-amino-2-methylpentane citrate, AMP citrate, 1,3-dimethylbutylamine citrate, 4-amino-2-pentanamine, Pentergy, and 4-AMP. The Google search engine was used to identify supplements meeting the inclusion criteria.

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1.3-dimethylbutylamine (DMBA) 1,3-dimethylamylamine (DMAA)

Figure 1. The chemical structures of DMBA and DMAA. Note that DMBA has one chiral center and that DMAA has two chiral centers.

Experimental

Materials

Two containers of each product were purchased. One container was analyzed by NSF International (Ann Arbor, MI, USA) and one container was analyzed by the Netherland's National Institute for Public Health and the Environment (RIVM).

NSF obtained the DMBA reference standard, heptafluorobutyric acid (ion chromatography grade) and ammonium formate (LC-MS grade) from Sigma-Aldrich (St Louis, MO, USA). Acetonitrile (LC-MS grade) was obtained from JT Baker (Center Valley, PA, USA). Water (Optima, LC-MS grade) for UHPLC was obtained from Fisher Scientific (Fairlawn, NJ, USA).

RIVM obtained a DMBA reference standard from Sigma-Aldrich (Zwijndrecht, the Netherlands). Formic acid (p.a.), acetonitrile (p.a.) and ammonium hydroxide (p.a.) were obtained from Merck (Darmstadt, Germany). Water was demineralized and filtered using a Millipak®200 0.22 μm filter from Millipore B.V. (Amsterdam, the Netherlands).

Instrumentation

At the NSF International laboratory, samples were analyzed using a QuattroMicro with an Acquity UPLC (Waters, Milford, MA, USA) fitted with a BEH C_{18} column (2.1 mm ID x 50 mm, 1.7 μm particle size). The flow rate was 0.5 mL/min with a column temperature of 40 °C. An injection volume of 5 μL was used. The mobile phase consisted of component A, 10 mM ammonium formate and 5 mM heptafluorobutyric acid (HFBA) in de-ionized water and component B, acetonitrile. A gradient elution was employed as follows: 0–2 min 100% A, 6–11 min 92% A, 11.1–15 min 10% A. The column was re-equilibrated for 4 min between runs. Tandem mass spectrometry was performed using electrospray ionization in positive polarity under the following conditions: capillary of 3.5 kV, cone of 19 V, extractor of 3 V, RF lens of 0.2 V, source temperature of 130 °C, desolvation temperature of 500 °C, desolvation gas flow of 750 L/hr and a collision energy of 9 eV. The precursor ion was *m/z* 102 and the product ions were *m/z* 85, 57 and 43 in multiple reaction monitoring (MRM) mode. A stock standard was prepared at 2.4 mg/mL in methanol. This standard was diluted to 1000 ng/mL in a solution of 50% 100 mM ammonium formate and 50 mM HFBA in de-ionized water and 50% methanol. Quantitation was performed using the product ion *m/z* 85. External standard method calibration was used since an isotopically labelled reference compound could not be commercially found. A 6-point linear calibration curve with $1/x^2$ weighting was utilized, with a linear range of 10-500 ng/mL. The presence of DMBA in the tested products was confirmed by comparison of the retention time and product ions to that of a reference standard.

At the RIVM laboratory samples were analyzed using a Waters Acquity™ ultra-performance liquid chromatography (UPLC) system fitted with an HSS C18 column (150 mm x 2.1 mm i.d., 1.8 μm; Waters Chromatography B.V., Etten-Leur, the Netherlands). Detection of the analytes was carried out using a Waters Synapt™ G2 quadrupole time of flight (QTOF) mass spectrometer (Waters Chromatography B. V., Etten-Leur, the Netherlands) with a Z-spray electrospray ionization (ESI) source operating in the positive ion mode. The instrument was tuned and calibrated in the mass range of 50–1200 Da using sodium formate in resolution mode (≥20 000 FWHM). Exact mass measurements of DMBA were based on the protonated molecules $[M+H]^+$. Leucine enkephalin (1 μg/mL) was used as lock mass standard after instrument calibration. Chromatographic and mass data were acquired and analyzed using Waters MassLynx v4.1 software.

The flow rate was 0.4 mL/min with a column temperature of 50 °C. An injection volume of 10 μL was used. The mobile phase consisted of mixture of component A: 5 mM ammonium formate, adjusted to pH 3.0 using formic acid and component B: 0.1% formic acid in acetonitrile (87:13). A gradient elution was employed as follows: 0–0.50 min 13% B, 0.50–10.00 min 50% B, 10.75 min 95% B, 10.75–12.25 95% B, 12.25–12.50 min 13% B, 12.50–15.00 min 13% B (column re-equilibration).

Tandem mass spectrometry (MS/MS) was performed using electrospray ionization in positive polarity using the resolution mode (≥20 000 FWHM) under the following conditions: capillary of 3.0 kV, cone of 20 V, source temperature of 120 °C, desolvation temperature of 150 °C, desolvation gas flow of 600 L/hr and a collision energy of 6 eV and collision energy ramp of 15–50 eV. The precursor ion was observed at *m/z* 102.1283 and the product ion was observed at *m/z* 85.1017. A stock standard was prepared at 0.3 mg/mL in methanol and diluted to 30 and 60 μg/mL in a mixture of component A and B (87:13). The presence of DMBA in the tested products was confirmed by comparison of the retention time, MS and MS/MS spectra to that of a reference standard.

Extraction method

Samples were prepared at NSF International in triplicate. One gram of powdered bulk product or the contents of 1-2 capsules were extracted with 20 mL of a methanol: water (50:50) solution by shaking for 20 min followed by sonication for 20 min. The resultant mixture was centrifuged at 5525 g for 20 min. The supernatant was reserved and filtered through a 0.45 mm PVDF syringe filter. The resulting solution was serially diluted by a factor of 10 from $1\text{-}10^2$ to $1\text{-}10^5$ with a mixture of deionized water containing 100 mM ammonium formate and 50 mM heptafluorobutyric acid, and methanol (50:50).

Samples were prepared for analysis at RIVM in duplo. Half a serving size of homogenized powdered bulk product or half the contents of 1 capsule were extracted with 50 mL of methanol by shaking for 5 min followed by sonication for 15 min. The resultant mixture was centrifuged at 3000 g for 15 min. The resulting solution was diluted 50x with a mixture of component A: 5 mM ammonium formate, adjusted to pH 3.0 using formic acid and component B: 0.1% formic acid in acetonitrile (87:13). All samples and solutions were filtered before use over a 0.2 μm filter (Spartan 30, Whatman GmbH, Dassel, Germany).

Results and discussion

Fourteen supplements met our inclusion criteria (Table 1). One of the 14 supplements, 'Frenzy' manufactured by Driven Sports, did not arrive from its US distributor and was instead purchased from an online supplement retailer in the UK. Supplements were labelled as sports supplements (38.5%; 5/13), weight loss supplements (38.5%; 5/13) and brain enhancers (23.0%; 3/13). The product category of one supplement was not discernable. Supplements were labelled as containing 4-amino-2-methylpentane citrate (85.7%; 12/14), AMP

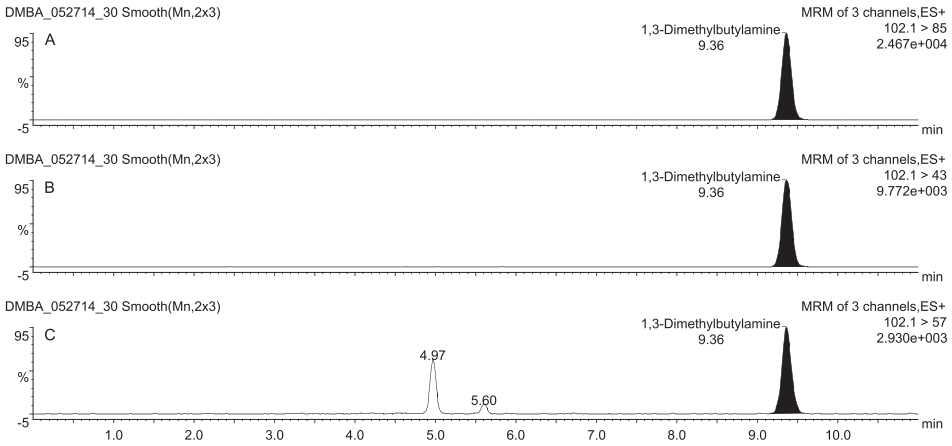

Figure 2. Product ion chromatograms of a sample extract. A) Quantifier MRM transition *m/z* 102.1 to *m/z* 85. B) Qualifier MRM transition *m/z* 102.1 to *m/z* 43. C) Qualifier MRM transition *m/z* 102.1 to *m/z* 57. The peaks at retention time 4.97 and 5.60 minutes were not investigated because it was not within the scope of this study.

citrate (7.1%; 1/14) or 4-amino-methylpentane citrate (7.1%; 1/14). No label provided the quantity per serving. Two supplements (14.3%; 2/14) described DMBA as if it were extracted from Pouchong tea.

DMBA was detected in 12 supplements (85.7%; 12/14) in the range of 13 to 120 mg DMBA per serving (as the free base). An example of the product ion chromatograms from a sample extract is illustrated in Figure 2. DMBA was not detected in two supplements. Following recommendations on the label for maximum daily intake, customers would consume from 0 to 320 mg of DMBA. For the 12 supplements that contained DMBA, the maximum daily intake ranged from 26 to 320 mg (Table 1).

Two supplements' labels ('Frenzy' and 'OxyphenXR AMP'D') implied that DMBA was extracted from Pouchong tea. Leaves from *Camellia sinensis* are used to prepare green, Pouchong and Oolong tea. The difference is the manner in which the leaves are prepared for each tea variety. Slight fermenting is involved in the preparation of the leaves prior to brewing Pouchong tea and, theoretically, could introduce compounds not found in green or Oolong tea.[13]

One study in Chinese by Chen and Ou purports to have found DMBA at levels of 0.012 ppm in Pouchung tea as a degradant upon storage; however, an authentic chemical reference standard was not used to confirm the identity or quantity of DMBA in this study.[14] Even if DMBA were found at these very low levels, manufactures would require at least 1000 kg of Pouchung tea to extract 12 mg of DMBA, and humans would not have previously been exposed to the high levels of DMBA that we found in dietary supplements. The only other study that we are aware of that purports to find DMBA in nature is a study in Chinese which analyzed the essential oil from the oleoresin of the Plains coreopsis (*Coreopsis tinctoria*), but the authors did not use a reference standard to confirm the finding.[15]

We are unaware of any scientific evidence that DMBA has ever been extracted from any plant, while synthetic DMBA is easy to synthesize and widely available.[16] DMBA belongs to the family of pressor-amines including the medicines DMAA, tuamine, and propylhexedrine.[8] The concentrations found in the supplements analyzed strongly suggest that DMBA is synthetically mass-produced to create pharmaceutical effects. It would appear to be very difficult, if not impossible, to justify the inclusion of DMBA in any nutritional supplement.

The health risks from DMBA in pharmacological doses are unknown. DMBA has never been studied in humans and its physiologic effects, to our knowledge, are only briefly mentioned in two small animal studies from the 1940s. One study examined DMBA's effect, along with several other aliphatic amines, on an undisclosed number of pithed cats and another study examined its effect on five pithed dogs.[17,18] These two small animal studies provide preliminary evidence that, as its structure would suggest, DMBA has pressor effects and is somewhat less potent than DMAA. DMBA should therefore be considered an active pharmaceutical ingredient that requires rigorous clinical testing and evaluation prior to marketing. Unfortunately, unless the laws regulating dietary supplements are reformed to prohibit unproven claims such as 'enhances athletic performance' or 'effective for weight loss' – additional aliphatic amines such as 2-amino-5-methylhexane, 2-aminohexane and 2-amino-6-methylheptane are likely to be introduced as soon as regulatory bodies begin to move against DMBA.

Recently, the Netherland's National Institute for Public Health and the Environment found DMBA in a supplement named 'Unstoppable' sold by Dedicated. The supplement did not have a marketing name for DMBA on its label. The 'Unstoppable' sample was obtained from a user who had reported adverse effects to the Netherlands Anti-Doping Authority. So far, three cases of adverse effects were reported for this supplement. The symptoms were similar and included the feeling of rushing, difficulty sitting still, a sense of motion and increased focus. It is not known if DMBA was responsible for the adverse effects noted by consumers. However, analysis of 'Unstoppable' demonstrates that DMBA may be present in supplements that do not list the ingredient on the label. (The implicated supplement was not part of our current study because it did not meet our inclusion criteria.)

In addition to adverse health effects, DMBA poses significant risk to the career of athletes: the World Anti-Doping Agency (WADA) will probably consider DMBA to be a prohibited substance under section S6.b because of its similarity with tuamine.[19]

Our study has several limitations. The sample size is too small for the quantitative findings to be representative. Nevertheless, quantitative data provide initial estimates of the amount of DMBA to which consumers might be exposed. In addition, the number of consumers exposed to DMBA supplements is unknown. We do not have any information regarding sales of

Table 1. Quantity of 1,3-dimethylbutylamine (DMBA) in dietary supplements

Supplement name (Manufacturer)	Retailer	Product Category	Manufacturer's name for DMBA provided on the supplement label	Recommended Serving size	Maximum daily intake	DMBA per serving	DMBA per maximum recommended daily intake
Contraband (Iron Forged Nutrition)	TGB SUPPLEMENTS.com	Sports nutrition	AMP Citrate	1 scoop (11.6 g)	1 scoop (11.6 g)	50 mg	50 mg
Redline White Heat (Vital Pharmaceuticals Inc)	Vpxsports.com	Sports nutrition	4-Amino-2-Methylpentane Citrate	1 scoop (4 g)	1 scoop (4 g)	76 mg	76 mg
Evol (Genomyx LLC)	AMAZON (sold by Fitness Factor)	Sports nutrition	4-Amino-2-Methylpentane Citrate	1 scoop (4.4 g)	2 scoops (8.8 g)	13 mg	26 mg
Preamp by Hybrid (DSEO, LLC)	BEST PRICE NUTRITION.com	Sports nutrition	4-Amino-2-Methylpentane Citrate	1 scoop (5 g)	2 scoops (10 g)	-*[#]	-*[#]
MD2 Meltdown (Vital Pharmaceuticals Inc)	Vpxsports.com	Weight loss	4-Amino-2-Methylpentane Citrate	2 capsules	3 capsules	110 mg	160 mg
OxyphenXR AMP'D (Beta Labs, LTD)	Myvitastore.com	[¤]	4-Amino methylpentane Citrate from Pochung Tea	2 capsules	3 capsules	86 mg	130 mg
OxyTHERM Pro (deNOVOLABS)	DPS NUTRITION.net	Weight loss	4-Amino-2-Methylpentane Citrate	1 capsule	2 capsules	39 mg	78 mg
AMP Citrate (Genomyx LLC)	A1 SUPPLEMENTS.com	Brain enhancers	4-Amino-2-Methylpentane Citrate	2 capsules	3 capsules	-*[§]	-*[§]
Oxyfit Xtreme (Oxyfit Xtreme)	PLANETARY NUTRITION.com	Weight loss	4-Amino-2-Methylpentane Citrate	1 capsule	2 capsules	53 mg	110 mg
Synetherm (Synetherm)	PLANETARY NUTRITION.com	Weight loss	4-Amino-2-Methylpentane Citrate	1 capsule	2 capsules	53 mg	110 mg
AMPitropin (Lecheek Nutrition)	AMAZON (Lightning Liquidators)	Brain enhancers	4-Amino-2-Methylpentane Citrate	1 capsule	3 capsules	110 mg	320 mg
Decimate Amplified (Genomyx LLC)	AMAZON (sold by Fitness Factor)	Weight loss	4-Amino-2-Methylpentane Citrate	1 capsule	2 capsules	49 mg	98 mg
AMPilean (Lecheek Nutrition)	AMAZON (sold by Fitness Factor)	Weight loss	4-Amino-2-Methylpentane Citrate	2 capsules	2 capsules	120 mg	120 mg
Frenzy (Driven Sports)	Driven Sports (sold by Predator Nutrition)	Sports nutrition	4-Amino-2-methylpentane Citrate from Pouchung Tea	1 scoop (8.1 g)	2 scoops (16.2 g)	110 mg	210 mg

[¤] Product category not discernible from the label;
* LOD of 1.5 mg/g (RIVM);
[#] LOD of 0.2 μg/g (NSF);
[§] LOD of 20 μg/g (NSF)
NSF = NSF International
RIVM = Netherland's National Institute for Public Health and the Environment

the analyzed supplements. Furthermore, we did not include DMBA supplements sold exclusively by distributors outside the US nor did we analyze supplements without a trivial/marketing name for DMBA on the label. Therefore, the number of supplements containing DMBA is likely much greater than suggested by our current analysis (as exemplified by finding DMBA in Unstoppable). Lastly, we did not test these supplements for caffeine and other stimulants that might heighten their risks.

Conclusion

Our objective was to determine if a stimulant never before sold for human consumption, DMBA, was present in dietary supplements sold by US distributors. We found that at least a dozen supplements sold by US distributors contain DMBA in dosages from 13 to 120 mg per serving. Given the potential health risks of untested pharmacologic stimulants, we strongly recommend that manufactures immediately recall all DMBA containing supplements. The FDA and other regulatory bodies should, without delay, warn consumers about the presence of DMBA in dietary supplements and clarify the legal status of DMBA. Until consumers can be assured that sports, weight loss and mind enhancing supplements do not contain untested pharmaceutical drugs, these products should be avoided.

Conflicts of interest

Drs Cohen & Venhuis have no conflicts of interest. Mr. Travis is an employee of NSF International. Some of NSF International's clients are dietary supplement manufacturers.

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