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Trabalho Complementar apresentado à Universidade Fernando Pessoa como parte dos requisitos para obtenção do grau de licenciado em Ciências da Nutrição

Orientador:

Professor Doutor João Paulo Soares Capela

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Abbreviations List

ANSES –- National Agency for Food, Environmental and Occupational Health Safety (from the french: "*Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail"*)

BfR – German Federal Institute for Risk Assessment

EFSA – European Food Safety Authorit

N.A – Not available

SD – Standart deviation

95% CI – 95% confidence interval

FDA - Food and Drug Administration

Resumo

Introdução: Cafeína e sinefrina são substâncias normalmente incluídas em produtos para perda de peso devido aos alegados efeitos termogénicos.

Objetivos: O objetivo deste trabalho de investigação é pesquisar a quantidade de cafeína e sinefrina encontrada em suplementos dietéticos disponíveis em websites. Por outro lado, compreender os efeitos adversos da ingestão de suplementos contendo cafeína e sinefrina para perda de peso. A questão básica é: quais são os efeitos adversos das quantidades de sinefrina e cafeína encontradas nos suplementos dietéticos?

Materiais e Métodos: Foi realizada uma procura exaustiva em plataformas que vendem suplementos desportivos e foram analisados os rótulos. Os critérios de inclusão foram suplementos que continham ambos os componentes, sinefrina e cafeína, independentemente da quantidade e do uso. Os critérios de exclusão foram se apenas um dos componentes do suplemento estivesse presente e se a fórmula e a quantidade de ingredientes não estivesse totalmente descrita no rótulo. Para a investigação de suplementos, foram considerados sítios da internet como os da Prozis, Eunutrition e Myprotein, dos sites NutriTienda, Fitnis e PlanetaHuerto.

Resultados e Discussão: Dentro dos critérios de inclusão e exclusão estabelecidos, foram encontrados 20 suplementos. Desses 20 apenas 3 (15%) dos 20 suplementos encontrados continham exclusivamente sinefrina, os restantes, 17 (85%) suplementos continham sinefrina na forma de extrato de laranja amarga (*Citrus aurantium*). Em relação à dose diária recomendada de sinefrina, o valor mínimo encontrado foi de 6 mg, como o mínimo de *citrus aurantium*, e o valor máximo, de 450 mg, já para o *citrus aurantium* o máximo foi de 840 mg. Em relação à cafeína, o valor mínimo encontrado foi de 100 mg e o valor máximo encontrado foi de 10000 mg.

Conclusões: Diversos suplementos possuem quantidades de sinefrina e cafeína que excedem o recomendado pelas agências de saúde de segurança. As informações em humanos ainda são escassas, e serão necessários mais estudos, com um tamanho amostral maior de indivíduos, bem como por um período maior de tempo para verificar a segurança da sinefrina combinada com a cafeína.

Palavras-Chave: Cafeina; sinefrina; *Citrus aurantium;* suplementos dietéticos; perda de peso; toxicidade cardiovascular;

Abstract

Introduction: Caffeine and synephrine are substances normally included in weight loss products due to claimed thermogenic effects.

Objectives: The objective of this research work was to search the amount of caffeine and synephrine found in dietary supplements available on websites. On the other hand, understand the adverse effects of intake of supplements containing caffeine and sinephrine for weight loss. The basic question was, what are the possible adverse effects of the amounts of synephrine, and caffeine found in dietary supplements?

Materials and Methods: An exhaustive search was carried out on platforms selling sports supplements and the labels evaluated. The inclusion criteria were supplements that contained both components, synephrine and caffeine, regardless of quantity or use. The exclusion criteria were if only one of the components was present, and if the formula and ingredients amount was not fully described on the label. For the investigation of supplements, websites such as Prozis, Eunutrition, Myprotein, NutriTienda, Fitnis and PlanetaHuerto were considered.

Results and Discussion: Within the established inclusion and exclusion criteria, twenty supplements were found. Of the 20 supplements, only 3 (15%) contained exclusively synephrine, meanwhile the remaining 17 (85%) contained synephrine in the form of bitter orange extract (*Citrus aurantium*). Regarding the recommended daily dose of sinephrine, the minimum value found was 6 mg, as was the minimum of citrus aurantium. The maximum value of sinephrine found was 450 mg, while for citrus aurantium the maximum was 840 mg. Related to caffeine, the minimum value found was 100 mg and the maximum value found was 10000 mg.

Conclusions: Several supplements have amounts of synephrine and caffeine that exceed the recommended by security health agencies. The information in humans is still scarce and more studies are needed with a larger sample of individuals, as well as for a longer periods of time to access the health safety of synephrine combined with caffeine.

Keywords: Caffeine, synephrine; Citrus aurantium; dietary supplements; body weight; cardiovascular toxicity.

Introduction

Caffeine and synephrine are substances normally included in weight loss products due to claims of promoting a thermogenic effect, or in other words, to stimulate the metabolism (1,2). Thermogenic food supplements claim increasing caloric expenditure (3).

Caffeine is an alkaloid that is found in varying amounts in the grains, leaves and fruits of more than 60 plants. Roasted coffee beans and tea leaves are the world's leading sources of caffeine in the diet (1). This substance contributes with positive effects on sports performance, as it increases attention and surveillance status, slows the feeling of tiredness, increases the time-trial of performance, can increase performance by approximately 3% in anaerobic exercises, increases the rate of glycogen synthesis and has no impact on hydration status. Caffeine is quickly absorbed through the gastrointestinal tract, and it is metabolized by the liver. The higher concentrations are about one hour post ingestion. This substance is effective in trained athletes when consumed in low to moderate doses (~3-6 mg/kg), because when consumed in higher doses doesn't result in performance (>9 mg/kg) (4). Supplementation with caffeine is often taken to increase lipolysis, fat oxidation and even lower glycogen breakdown (3).

Synephrine is a derivative of phenylethylamine and a sympathomimetic agent that can be used to stimulate specific adrenergic receptors (β3, but not β1, β2 or α1) that stimulate fat metabolism without any side effects that are normally associated with other compounds that boost adrenergic receptors (3). Synephrine is naturally present in bitter orange (Citrus aurantium) and other citrus species. Citrus aurantium is a known fruit that is commonly used as a herbal medicine to treat digestive problems is Asia. Can be also used as a mild stimulant, contributes to appetite suppression and increased metabolic rate and lipolysis (3). In some studies, acute p-synephrine intake (~0.5 mg/kg) proved effective to increase energy expenditure and lipolysis at rest, probably due to its high binding affinity to β3 adrenoreceptors (5,6).

In a clinical trial, the intake of p-synephrine (3 mg/kg) revealed the ability to increase the rate of fat oxidation during exercise, without significantly affecting exercise energy expenditure or heart rate (1).

This component is identified as "the active component" for products onto weight loss containing bitter orange or Citrus aurantium (7). Synephrine can be found in the human organism, where it is considered a vestige amine due to its low plasmatic levels. Synephrine exists in three different positional isomers o-, p- and m-, but only p- and m-

synephrine have been described in weight-loss products (7). These isomers differ in the position of the hydroxyl group in the phenolic group, which alters their pharmacodynamic characteristics. p-Synephrine is widely used in combination with caffeine and other ingredients in products designed to support weight management (7). It becomes one of the most popular stimulants found in weight loss products, since the US Food and Drug Administration (FDA) banned ephedrine containing dietary supplements.
Given its use in dietary supplements several health and food authorities have issued recommendations on the levels deemed safe for human use. In Table 1 are included several reference values for synephrine and caffeine consumption.

In the past few years, the problems associated with overweight, and obesity have been getting worse. Thus, obese people are more likely to have a higher incidence of hypertension, hyperlipidemia, insulin resistance and glucose intolerance. With this, the market for dietary supplements for weight loss has also been increasing, since these individuals want to get results easier and faster (8).

Table 2:Safety authorities' recommendations for synephrine and caffeine consumption

Reference	Institution	Document	Synephrine	Caffeine	Synefrine + Caffeine
(9)	France/ ANSES	Dietary supplements for weight loss containing p-synephrine	- The Agency considers that intake levels of p-synephrine through food supplements must remain below 20 mg/day. - Not taking p-synephrine-based weight-loss supplements along with caffeine, and strongly discourages taking such products during physical exercise, as well as their use by sensitive groups, including individuals following medical treatments, pregnant or breastfeeding women, children and adolescents.	N.A	N.A
2003 (10)	New Zealand Medicines and Medical Devices Safety Authority (MedSafe)	Committees "Minutes of the 30th meeting of the Medicines Classification Committee - 26 November 2003"	- Recommended maximum dose of 30 mg / day of synephrine.	N.A	N.A
(11)	Health Canada	"Guidelines for the use of Synephrine in Natural Health Products"	- Maximum dose of 30 mg/day; - Caffeine is not allowed in products that contain synephrine.	- Healthy adults: No more than 400 mg of caffeine per day - For women of childbearing age the recommendation is a maximum daily caffeine intake of no more than 300 mg	N.A
(12)	Health Canada	"Synephrine, Octopamine and Caffeine, Health Risk Assessment (HRA) Report"	- Maximum doses of 50 mg / day of p-synephrine when not combinate.	N.A	- Maximum dose of 40 mg of p-synephrine in combination with maximum dose of 320 mg /day of caffeine in healthy adults
(13)	German Federal Institute of Risk Assessment (BfR)	Opinion "Health assessment of sports and weight loss products containing synephrine and cafeine"	- Maximum of 6.7 mg / day in food supplements, ensuring that in conjunction with ingestion of foods containing synephrine, it does not exceed 25.7 mg / day (95th percentile of regular consumers of foods containing synephrine).	N.A	N.A
(14)	Europe/ EFSA	EFSA explains risk assessment	N.A	- Intakes up to 400mg per day (about 5.7mg / kg bw per day) consumed throughout the day do not raise safety concerns for healthy adults in the general population, except pregnant women (no more than 200 mg/day).	N.A

Methodology

In an early stage of research, intensive investigation was carried out on platforms selling sports supplements. Supplement analysis was made based solely on the ingredient contents descried in the product label. For the selection of dietary supplements within this research, inclusion and exclusion criteria were established. For the inclusion criteria, only supplements with both components, synephrine (either pure or in the form of *Citrus aurantium* extract) and caffeine, regardless of quantity and use. As for the exclusion criteria, supplements were not included if only one of the components was in the label, or the formula and ingredients amount was not fully described on the label.

The information contained on the labels of the supplements, was taken from the websites of the company's Prozis companies (www.prozis.pt), Eunutrition (eunutrition.com), Myprotein (pt.myprotein.com/), NutriTienda websites (www.nutritienda.com / en), Amazon (www.amazon.com), Fitnis (www.fitnis.pt) and PlanetaHuerto (www.planetahuerto.pt). These websites were chosen based on the fact that these are major players in selling supplements and could be shipped to Portugal. The ingredients disclosed in the label where thoroughly analysed and grouped. The search for supplements was conducted between October 2020 and November 2020. After the investigation, twenty supplements have been found that met the inclusion criteria.

In a later stage, the databases PubMed, Science direct and Google Scholar were used to search scientific articles for bibliographic support, with the keywords "Caffeine and synephrine, toxicology", "*Citrus aurantium* and caffeine, adverse cardiovascular*"*; "dietary supplements, body weight".

Results

The ingredients and respective quantities mentioned on the label, prepared by the producers and / or distributors of 20 food supplements containing synephrine and caffeine, are described in Table 2. The table describes different groups; synephrine or bitter orange extract (Citrus aurantium), caffeine, vegetable extracts with caffeine, other plant extracts, amino acids, micronutrients and finally others. It's important to mentioned that the amounts referred are for the recommended daily dose. It is relevant to say that

the energy and macronutrient value of these supplements is not available as they do not provide relevant calories, thus the caloric value is not listed in the label.

Table 2: Comparison of the content of ingridients containing synephrine and caffeine sources of different dietary supplements for weight loss containing synephrine and caffeine available in the websites. The amounts referred are for the recommended daily dose mentioned by the producer in the label.

Nº	Supplements (company)	Daily recommended dose	Synephrine or bitter orange extract (citrus aurantium)	Caffeine	Vegetable extracts with caffeine	Other plant extracts	Amino acids	Micronutrients	Others
1	Lipo 6 Black Ultra Concentrate 72 cáps (nutrex)	2 capsules	120 mg (*Citrus aurantium*)	360 mg	180 mg – guarana (containing 22% caffeine)	5 mg – piper nigrum. 5 mg – cayenne pepper.	L-tyrosine - 220 mg	Chromium - 200 mg;	-
2	ThermoCore™ 90 cáps (amix)	2 capsules	10 mg - Bitter orange extract (*Citrus aurantium*:95% synephrine)	160 mg	200 mg - guarana; 250 mg – green tea extract	*Coleus forskohlii* (10% *Forskohlii*) - 250mg; White Willow extract - 200 mg; Black pepper extract - 5 mg;	-	-	-
3	THERMO SPEED EXTREME (MEGA CAPSULES) (olimp nutrition)	2 capsules	20 mg (synephrine); 334 mg of *Citrus aurantium*	160 mg	182 mg - guarana extract (22%); 500 mg – green tea extract	Black pepper extract - 5 mg; Pioerin (95%) - 5 mg;	L-tyrosine: 1000 mg	-	-
4	GRENADE THERMO DETONATOR (Grenade)	4 capsules	840 mg (bitter orange extract)	450 mg	1000 mg – green tea; 20 mg – green coffee	-	Cayenne - 400mg; Phenylalanine - 50 mg;	-	-
5	CRANK (ESN)	1 spoon = 19g	6 mg - sinefrina (100 mg bitter orange extract)	300 mg	250 mg – green tea extract	Grape seed extract - 250 mg; Schizandra extract - 200 mg; Ginseng root extract - 100 mg; Rhodiola rosea extract - 100 mg; Pepper extract - 4.07 mg; including piperine 4 mg;	Citrulline malate - 6000 mg; Arginine alpha-ketoglutarate - 4000 mg; L-tyrosine - 1000 mg; L-glycine - 1000 mg; Taurine - 1000 mg; Glucuronolactone - 500 mg;	-	-
6	Termogénico Completo (BULK)	3 capsules	450 mg - synephrine	300 mg	300 mg – green tea extract	Bioperine black pepper extract - 5 mg; French ketones - 150 mg; Cayenne pepper - 150 mg;	L-tyrosine - 200 mg; L-thianine - 150 mg;	Chromium picolinate - 1 mg;	-
7	CITROLINEA MAX – 40 COMPRIMIDOS (ESI)	2 pills	30 mg - 6% Synephrine; 500 mg - bitter orange extract (Citrus aurantium)	10% caffeine - 100 mg;	Guarana (Paullilia cupana K.) caffeine - 400 mg; green tea (Camelia sinensis) - 300 mg; kola nut (Cola Nitida Schott); caffeine - 300 mg;	20% polyphenols -60 mg	L-carnitine - 200 mg;	-	-
8	Thermopure Boost (Myprotein)	4 capsules	125 mg (Citrus aurantium)	175.5 mg	450 mg – green tea extract	Siberian ginseng - 150 mg; 225 mg of extract equivalent to 900 mg of white beans; Thermogenic mixture (caffeine, black	-	Chromium - 118.8 µg; Vitamin B6 - 4.6 mg; Vitamin B12 - 24 µg	-

						ketones, powdered cayenne pepper, chocamine) - 825 mg;			
9	Pwd Redhell Essence 90 cápsulas (PWD Nutrition)	3 capsules	180 mg - Bitter orange extract (Citrus aurantium L.)	100 mg	300 mg - guarana; Kola nut (2,5% de caffeine) - 240 mg,	Grass mate - 135 mg, Fucus - 100 mg, Resveratrol 45 mg	Acetyl-L-Tyrosine 300 mg, 300 mg acetylcarnitine;	Anti-caking agent (magnesium stearate)	Bulking agent (maltodextrin); 27 mg of anti-caking agent (silicon dioxide); Capsule (gelatin and coloring (E171 and E172)
10	THERMO SHAPE 2.0 (Activlab)	3 capsules	300 mg - synephrine	200 mg	200 mg – green tea extract	EGCG (epigallocatechin gallate) - 90 mg; Cambodia garcinia extract - 150 mg; HCA - hydroxycitric acid: 90 mg; Cayenne pepper extract - 100 mg; Capsaicin - 0.3 mg; Black pepper extract - 5 mg; Piperine - 4.7 mg;	L-carnitine - 300 mg; L-tyrosine - 100 mg;	Chromium – 40 µg;	-
11	Thermonex 120 capsules (BSN)	3 capsules	20 mg - synephrine	250 mg	375 mg – green tea extract	Green mate – 20 mg	Octopamine HCL - 200mg; Evodiamine - 40 mg; L-Tyrosine - 300mg; Naringin - 50mg; Diiodotyrosine - 100mcg	-	Iodotyrosine - 100mcg
12	Lipo 6 Black Hers - 120 capsules (nutrex)	3 capsules	40 mg - Citrus aurantium	200 mg	-	Cocoa Theobroma (grain) - 208.5 mg; *Coleus forskohlii* (Root) (containing Forskolin) - 25 mg; Teacrina (as TeaCrine®) - 50 mg;	-	Iodine - 75mcg; Vitamin D - 100 IU; Folic acid - 100 mcg; Vitamin B12 - 1.25mcg; Chromium - 20 mcg;	Glycerin, water, hypromellose (vegetable capsule), titanium, dye: Allura Red (E129), Bright blue FCF (E133), Yellow (E110).
13	LIPO TONE UP ÉVOLUTION (EU nutrition)	3 capsules	100 mg - Bitter Orange extract (*Citrus aurantium* 6% synephrine)	200 mg	100 mg - guarana; 200 mg – green tea extract; dry extract of kola nut - 300 mg;	Black pepper extract - 9 mg; White Willow Bark Extract - 150 mg; dry extract Evodia - 100mg; Powdered melon - 100 mg; Dry extract of bauhinia - 21mg; Dry extract of garcinia (*garcinia cambogia*) - 40mg, Dry extract pillosina cnidium - 21mg;	L-tyrosine - 100 mg	Chromium picolinate - 200mg;	-
14	Machine Man Burner 120 caps (Activlab)	4 capsules	335 mg bitter orange extract	156 mg	200 mg – guarana; 223 mg – green tea extract;	5.30 mg black pepper extract; 100 mg cayenne peppe; 300 mg of Dandelion (*Taraxacum officinale*), 300 mg of Horsetail Extract (*Equisetum arvense*) (whole plant), 300 mg of *Fucus vesiculosus* (extract) (with 300 µg of Iodine)	1000 mg L-Carnitine, 1000 mg Taurine, 400 mg L-Tyrosine, 300 mg of L-Tryptophan, 200 mg of L-Phenylalanine	32 mg of Vitamin B3 (Niacin), 12 mg of Vitamin B5 (Pantothenic Acid), 2.80 mg of Vitamin B2 (Riboflavin), 2.80 mg Vitamin B6 (Pyridoxine), 2.20 mg of Vitamin B1 (Thiamine), 400 µg of Vitamin B9 (Folic Acid), 100 µg of Vitamin B7 (Biotin), 5 µg of Vitamin B12 (Cyanocobalamin), 400 µg chromium	400 mg of FOS (Fructooligosaccharides), 400 mg of Inulin, 400 mg of Chitosan, 200 mg of Alpha Lipoic Acid (ALA), 100 mg of Inositol

15	Stack Fire Plus (+WATT)	2 capsules	12 mg (synephrine); 200 mg (bitter orange extract)	200 mg	100 mg green tea	84 mg (garcinia cambogia); 100 mg cayenne); 184 mg of *cocoa extract* (Theobroma cacao) (seed), 60 mg of *Fucus vesiculosus* (with 0.2% Iodine), 40 mg of *Coleus forskohlii* (root) (with forskolin)	L-carnitine 100 mg	80 mg Vitamin C; 12 mg Vitamin E;	100 mg alpha lipoic acid (ALA)
16	Thermo Shape Man 120 caps (activlab)	3 capsules	300 mg bitter orange extract	200 mg	-	-	500 mg L-Tyrosine;	4.2 mg Vitamin B6; 15 µg Vitamin D; Pantothenic acid (vitamin B5) 18 mg 300%; Zinc 30 mg 300%	Sodium D-aspartate 1500 mg; D-aspartic acid 1150 mg
17	Lipolean 90 Caps (Amix)	2 capsules	10 mg - *Citrus aurantium* (Standardized by 6% Synephrine)	200 mg	4 mg guarana extraxt; 8 mg green tea extract;	5 mg Black pepper; 60 mg Capsicum Extract, 20 mg White Willow Extract (*Salix alba L.)* (bark) (with 15% Salicin), 6 mg Ginger Extract (*Zingiber officinale*) (root) (with 20% gingeroles)	54 mg L-Carnitine; 10 mg L-Tryptophan; 50 Taurine;	2.8 Vitamin B6	530 mg of conjugated linoleic acid (CLA)
18	Lipolean Man Cut Packs (Amix)	1 pack	10 mg *Citrus aurantium* (6% of synephrine)	200 mg	4 mg Guarana; 8 mg green tea extract;	250 mg *Garcinia cambogia*; 60 mg Capsicum Extract, 20 mg White Willow Extract (Salix Alba l.) (With 15% Salicin), 6mg Ginger Extract (*Zingiber officinale*) (with 20% gingeroles)	604 mg L-Carnitine, 550 mg Taurine, 100 mg L-Methionine, 10 mg L-Tryptophan,	4.3 mg of Vitamin B6 (Pyridoxine)	550 mg Choline Bitartrate, 550 mg Inositol, 250 mg Betaine HCL
19	LIPO TONE UP SUPER WOMAN (EU Nutrition)	3 capsules	400 mg Dry bitter orange extract	200 mg	60 mg guarana; 100 mg green tea esxtract;	6mg Cayenne; 6 mg black pepper; 100 mg of cocoa Extract (*Theobroma cacao*), 100 mg of Maca Extract (Lepidium meyenii walpers), 60 mg of Giseng Extract (Panax giseng camey), 51 mg of Dandelion (*Taraxacum officinale*) (extract), 21 mg of Cinnamon (*Cinnamomum verum* J. Presl) (powder)	400mg Acetyl L-Carnitine, 100mg L-Arginine	100mg of Vitamin B1 (Thiamine), 21mg of Vitamin C, 0.9mg of Vitamin B6 (Pyridoxine); 200 µg chromium	100 mg collagen, 45 mg coenzyme Q10
20	Ripped Freak (PharmaFreak)	2 capsules	30 mg bitter orange (*Citrus aurantium*)	350 mg	Green tea: 200 mg (leaf) + 140 mg GTC + 90 mg EGCG	200 mg Cayenne; 60 mg of *Olea europaea* (leaf)	-	80 mg of Vitamin C, 1.40 mg of Vitamin B6 (Pyridoxine), 5.0 µg of Vitamin D	-

Of the 20 supplements containing caffeine and synephrine or Citrus aurantium, only 3 (15%) of the 20 supplements contained pure synephrine in its composition, as the

remaining 17 (85%) contained synephrine in the form of bitter orange extract (Citrus aurantium).

Regarding the daily recommended dose synephrine, the minimum value found was 6 mg, corresponding to the supplement number 5, and the maximum value was 450 mg, corresponding to number 6. Also, supplements may contain standardized extracts of Citrus aurantium for their synephrine content, which is normally at 6%, but one as 95%. Taking these values in mind one can calculate the amount of synephrine in those extracts. Thus, 6 supplements provide less than 100 mg, 4 provide between 100 mg and 200 mg and 7 supplements provide more than 200 mg of Citrus aurantium per daily dose.

Regarding the amount of caffeine and taking into account the daily recommended dose, the minimum value found was 100 mg which corresponded to supplement number 7 and 9, meanwhile the maximum value found was 450 mg, corresponding to number 4. In addition to caffeine, about 95% of supplements contained other combinations of plant extracts that contained caffeine, in particular green tea, green tea extract, guarana extract and kola nut. This addition renders impossible to calculate the exact amount of caffeine contained in those supplements.

With a total of 20 supplements found, the median was calculated, as well as the 95% confidence interval for caffeine, synephrine and bitter orange extract. Thus, the median for caffeine is 200 mg at a 95% confidence interval = 176 - 300 (Median ± 95% CI). For synephrine, there is a median of 25 mg at a 95% CI = 6 - 450 (Median ± 95% CI). As for the bitter orange extract, there is a median of 190 mg in a 95% CI = 100 - 400 (Median ± 95% CI) (Figure 1).

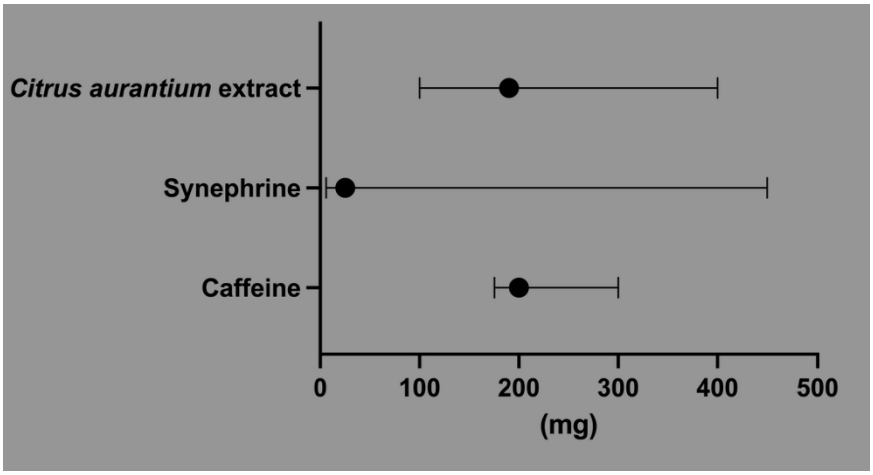

Figure 1: Graph plotting the median and confidence interval of caffeine, synephrine and synephrine in bitter orange extract in the twenty supplements analyzed. Values calculated according to the daily doses recommended by the manufacturer.

Beyond the most important components there are a few more plant extract ingredients that standed out including, black pepper extract, cocoa extract, cayenne extract, polyphenols for example. Also, contained amino acids, micronutrients and others that can be considered vestigial.

Furthermore, the nutritional information in the label or the description on internet website, sometimes included the following warnings: “Food supplements should not be used as a substitute for a varied and balanced diet, as well as a healthy lifestyle”, "Keep out of the reach of children", "Do not exceed the recommended daily dose", "This product is not intended to diagnose, treat, cure or prevent any disease", "The consumption of this product is contraindicated for people with physical or mental problems, those taking any medication, under the age of 18, pregnant women and those under medical supervision", "Avoid this product if you are allergic to aspirin or have high blood pressure, arrhythmia, glaucoma, seizures, ulcers, difficulty urinating, an enlarged prostate, diabetes, thyroid or eating disorders".

Discussion

A number of international safety authorities in different European Union (EU) Member States have carried out risk assessments or issued warnings for synephrine intended for weight loss and sport performance improvement such as its combination with caffeine (18). Of the organizations analyzed, it is worth noting that they present very different daily limits. As an example, the German Federal Institute for Risk Assessment (BfR) considered the maximum daily dose of 6,7 mg of synephrine in food supplements to be safe (BfR, 2012). In this study, only 2 supplements (number 5, 18) respect this recommendation, or about 80% of supplements exceed the limits imposed by this organization and may endanger the health of those who consume these products in concordance to that organization. By the Medsafe organization of New Zealand, the recommended dose is 30 mg. By the analyzed supplements, 8 fit these stipulated limits, therefore 60% exceed these recommendations (numbers 2, 3, 5, 7, 11, 15, 18, 20). In 2010 Health Canada established a maximum limit of 30 mg / day of synephrine, where it stated that supplements containing both components, synephrine with caffeine, were not allowed. About caffeine for healthy adults the ideal would be no more than 400 mg per day, for pregnant women the recommended is no more than 300 mg per day (11). However, in 2011, the same safety authority, Health Canada, mentioned that when synephrine is taken alone, a maximum dose of 50 mg was recommended, but when

combined with caffeine the maximum amount allowed is 40 mg synephrine or less in combination with a maximum of 320 mg of caffeine in healthy adults (11,13). For the first recommendation, given in 2010 by Health Canada, eight supplements fit the established limits, as in the recommendation imposed by Med safe. Meanwhile for the later recommendation of 2011, the limits for consumption of synephrine alone, nine supplements comply (about 45%). When the two ingredients are combined, only 8 comply with the established limits (numbers 2, 3, 5, 7, 11, 12, 15, 18). Of note, the real caffeine amount in several supplements is certainly higher, as several contained plant extracts that contained caffeine beyond caffeine itself. This addition will increase the total amount of the caffeine intake by the consumer, rendering impossible to the consumer to know the exact amount of caffeine included in the supplement. When talking only about caffeine consumption, EFSA recommends consumption up to 400 mg for healthy adults, but only up to 200 mg for pregnant women and breastfeeding women. Meanwhile, for children and teenagers the information is not enough to issue a recommended safe daily dose in these groups. Finally, in 2014, the institution ANSES concluded that the effects of p-synephrine alone are observed from around 20 mg, as with 50 mg there is an evident effect on heart rate and systolic and diastolic blood pressure and that Caffeine can potentiate the cardiovascular effects of synephrine. Thus, ANSES recommends avoiding combining p-synephrine with caffeine or preparations containing it (9). This agency also recognizes that the use of mixtures containing p-synephrine during physical exercise may modify blood pressure tolerance and increase acute cardiovascular risk (9). It is worth to mention that there are two supplements, number 6 and number 19, that has a high level of p-synephrine, 450 mg per dose and 400 mg per dose, respectively. Taking into account the high value of the amount of p-synephrine in these two over-the-counter products without the need for a prescription, they can have greater consequences for health given the high amounts of this compound.

Several studies in animals and humans were designed to assess the potential adverse effects and toxicity of synephrine. A study conducted by Arbo and collaborators, had the initiative to evaluate the subchronic toxicity of products containing Citrus aurantium / p-synephrine in mice. The mice were divided into six groups and received orally a dry extract of *Citrus aurantium* (containing 7.5% p-synephrine) 400, 2,000 or 4000 mg / kg or p-synephrine 30 or 300 mg / kg, for 28 consecutive days. There was an attenuation in the body weight gain of the animals treated with both doses of p-synephrine, what it means is that both synephrine and bitter orange extract have decreased

in weight. Organ weight, biochemical and hematological parameters were not changed in all treated mice. This study concluded that the results indicate a low subchronic toxicity of the ingredients tested in mice and a possible change in oxidative metabolism. However, more tests are needed to better elucidate the effects of these compounds on the antioxidant system (15).

Another study determined if relatively pure synephrine or synephrine present as a constituent of a bitter orange extract produced developmental toxicity in mice. Sprague-Dawley rats were dosed daily by gavage with one of eight doses of synephrine from one of two different extracts: vehicle (0.25% methyl cellulose); 1 mg, 2.5 mg, 5 mg, 10 mg and 25 mg/kg body weight using the p-synephrine extract; 10 mg and 25 mg/kg body weight using the bitter orange extract. Caffeine was added to some doses. At doses up to 100 mg synephrine / kg body weight, there were no adverse effects on embryopathy, fetal weight or incidence of visceral or skeletal abnormalities. A decrease in maternal weight was observed with the administration of 50 mg of synephrine / kg of body weight when administered as a 6% synephrine extract with 25 mg of caffeine / kg of body weight, as well as a decrease in maternal weight was detected in the group in which only caffeine was administered. This decrease in body weight was assumed to be a decrease in food consumption, which was observed in these two groups. Authors estimate that administering / consuming doses up to 100 mg synephrine / kg body weight did not produce developmental toxicity in Sprague-Dawley rats (16). This study in animals highlights the possibility that during pregnancy synephrine alone or in combination with caffeine could cause developmental problems to humans.

Haller and collaborators conducted a tree-arm randomized placebo-controlled crossover clinical trial involving 10 healthy men and women aged between 18-45 years. Subjects ingested one dose of dietary supplement with 21 mg synephrine and 304 mg caffeine, under resting conditions and 1 hour prior moderately intense exercise (30 min on cycle ergometer at 75–80% HRmax), with a placebo exercise control. The concentrations of both components were measure over 12 hours. In the end of the study no significant adverse effects occurred. Blood pressure and plasma glucose increased post-exercise with supplementation use. Exercise was perceived as less strenuous after dietary supplement, possibly due to the stimulant effects of caffeine (17).

A double-blind, placebo-controlled, randomized pilot study evaluated the thermogenic effects of p-synephrine alone and in conjunction with two flavonoids, naringin and hesperidin. Approximately 50 people participated and were about 8 to 10

hours fasted without consuming beverages with caffeine, nicotine, exercising or participating in vigorous physical activities (18). They were divided into 5 groups including a control group. After individuals were at rest for 10 to 15 minutes, resting metabolic rates (RMR), blood pressure, heart rate and the self-reported rating scale were determined at baseline and after 75 minutes the oral ingestion of the products in test. p-synephrine was administered in the form of the patented bitter orange extract Advantra Z® which contained 60% active synephrine. Naringin and hesperidin were 96% pure where they were administered together with Advantra Z® in different amounts (18). None of the groups showed changes in heart rate or blood pressure compared to the control group and the other groups that were targeted by "treatments" (18).

Another author in 2011 investigated the cardiovascular effects of a supplement that in its composition contained caffeine, bitter orange extract (p-synephrine) and green tea extract in individuals with excess weight or obesity grade 1 (19). Participated 14 women and 9 men and their BMI was 26.6 + - 3.8 kg / m. Participants arrived at the site on an overnight fast and went to consume 3 meals, where they would also ingest the placebo capsule or the food supplement with 13 mg of p-synephrine, 176 mg of caffeine in form of guarana extract and 55,5 mg of green tea extract and some ingredients in smaller amounts. The next day the process was repeated. From what was observed there were no effects on heart rate, systolic and diastolic blood pressure or mean arterial pressure. No between or within group differences were observed when data were analyzed for gender and caffeine usage. However, there was a slight decrease in the resting respiratory exchange rate in individuals who typically consume low amounts of caffeine in response to ingestion of the product containing caffeine and p-synephrine. Thus, authors concluded that the ingestion of a product with the combination of synephrine, caffeine and green tea extract does not lead to an increase in the negative cardiovascular effects, and that fat oxidation may increase in certain populations (19).

Sidney J. Stohs has long been devoted to studying bitter orange extract and p-synephrine. The author recently published a review of published case reports concerning adverse effects associated with multi-ingredient dietary supplements containing pine extract, bitter orange and p-synephrine (17). In this review, Stohs highlights the requirement of doing more quality-controlled and randomized studies, as well as double-blind and placebo-controlled studies, in order to avoid unsupported case reports, opinions and points of view, or extrapolations, which he thinks are dominant, when concluding about the safety and efficacy of dietary supplements, in spite of having not been

determined any direct link proven (20). As Stohs refers, “in some cases products were not being consumed as recommended, and it was not always clear whether the subjects were using other unreported dietary supplements and/or drugs” (20).

Furthermore, Stohs underlines the lack of revision of the existing published literature on some case studies, together with the lack of proves on the adverse effects at regularly used doses in several clinical studies. The author also stands out that in most cases there was no appropriate detail or no indication of the composition of the product involved or any analysis of the product itself. Finally, he stresses that the studies “do not demonstrate a direct relationship between bitter orange extract (p-synephrine) and the observed effect” (20).

Stohs states a case study of a subject which reportedly consumed a product containing 500 mg of a bitter orange extract (6% p-synephrine) and had a tachycardia episode afterwards. The experience was repeated by the authors one month later, but there could not be any clear conclusion on the results, since there was no complete information about the composition of the dietary supplement. As there was no identification of other possible responsible ingredients, it was “not clear whether the response was due to the bitter orange extract or some other ingredient or adulterant” (20). Stohs also raises another a case of a man with an ascending aortic dissection who consumed two doses of a supplement allegedly containing p-synephrine, but again without any other information about its composition: “The product may have contained 135 mg caffeine, 10 mg p-synephrine, 1.5 grams beta-alanine, Mucuna pruriens extract standardized for L-DOPA, and over 10 other ingredients” (20). He stresses that no scientific literature was reviewed, no reports or knowledge on p-synephrine amounts in the product were made and no adverse cardiovascular effects associated with p-synephrine were ever presented by controlled studies. Additionally, he says that “no cause-and-effect relationship” could be established by the authors between the product and any ingredients “including p-synephrine” and the resulting problem.

On another published case study of ST-segment elevation myocardial infarction (STEMI) of a 22 year-old man who consumed a sports performance product containing p-synephrine, caffeine, and other ingredients, Stohs refers the lack of information on the amounts of each ingredient in the product, in spite of having some information presented on labels, as was the case of caffeine. In this case study, he reports the failure of the authors to revise the literature, their erroneous assumption of the patient condition as a cause of the use of supplements containing p-synephrine, as well as the assumption of

adverse effects from mixing caffeine with p-synephrine, once there was no demonstration of any direct connection or association.

Conclusion

Many supplements have amounts of synephrine and caffeine that exceed those recommended by health safety agencies, which can put consumers' health at risk. In addition, not only can the combination of p-synephrine and caffeine components cause adverse cardiovascular effects, but other compounds can potentiate these problems, or even other medications taken at the same time for an illness. Also, individuals with known cardiovascular diseases who consume this type of supplement, and do not have this information, may be at greater health risk. In short, it is noteworthy that there is a lack of literature review and articles published on this topic, as well as the lack of scientific evidence of adverse effects.

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