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A randomized trial on the effects of CitrusiM® (*Citrus sinensis* (L.) Osbeck dried extract) on body composition

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SUMMARY

Objective: In this report, we investigated the effects of CitrusiM® (*Citrus sinensis* (L.) Osbeck dried extract) on body composition, defined in terms of two outcomes: percentage of lean mass and percentage of fat mass.

Research methods & procedures: A double-blind randomized experiment with overweight and obese volunteers was conducted in which the two variables were measured at baseline and three months afterwards. The treatment levels corresponded to three different daily doses of CitrusiM®: 0 mg (control), 0.5 g, and 1 g. All patients met with a nutritionist, and were given a standardized diet with their assigned treatments. At the end of three months, the intermediate outcomes of diet compliance (in percent) and amount of exercise (either none, low level, or high level) were recorded. The covariates that were collected for each subject were age, height, and sex. We analyzed the effects of the treatments on the two outcomes under a Bayesian framework of the Rubin Causal Model, and accounted for possible relationships between both diet compliance and exercise level and the three outcomes of interest in the analyses. Study registered under Research Ethics Committee of the Faculdade de Ciências Médicas

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Results: There was a statistically significant evidence that the treatment may increase lean mass and reduce fat mass.

Conclusion: A 3-month treatment with CitrusiM® (0.5 g or 1 g, daily) was able to increase lean mass, reduce fat mass, in the participants.

1. Introduction

In this report, we investigated the effects of *Citrus sinensis* (L.) Osbeck on body composition. Obesity is defined by the World Health Organization (WHO) as an accumulation of excessive or abnormal fat that can compromise health [1]. It currently constitutes a significant proportion of epidemiological issues globally, and can be considered the most important nutritional disorder. It afflicts individuals at a higher rate across all races and sexes [2] in both developed and developing countries [3]. It is estimated that in 2025, approximately 2.3 billion adults will be overweight, and over 700 million will be obese [1,2]. In addition, the majority of the world's population lives in countries where the number of deaths due to being overweight or obese is larger than the number of deaths due to malnutrition [1] because of the diseases associated with the former. Examples of such comorbidities are cardiovascular dysfunctions, diabetes, musculoskeletal disorders [1], and 13 types of cancers, such as esophagus (adenocarcinoma), stomach (cardia), pancreas, gall bladder, liver, intestine (colon and rectum), kidneys, breast (postmenopausal women), ovary, endometrium, meningioma, thyroid, and multiple myeloma [4].

For weight reduction, the WHO advises reducing carbohydrate and fat intake and increasing intake of vegetables, fruits, and fiber, as well as regular physical exercise [1]. However, some patients do not respond to such a strategy, and therefore pharmacological treatment may be used as a complement to the nutritional re-education [1,5].

In this context, natural products have been shown to be a valuable source of bioactive agents, playing a leading role in drug discovery [6]. Here we focus on the Moro blood orange fruit (*C. sinensis* (L.) Osbeck), which contains a variety of phytochemicals that contribute to its general flavor and biological properties. These substances include organic acids (mainly citric, malic, and isocitric), carotenoids (xanthophylls and carotenes), vitamins (A, B1, B3, B6 and C), aromatic compounds (esters, alcohols, ketones, lactones, and volatile hydrocarbons), hydroxycinnamic acids, and anthocyanins [7–9].

Recently, the study of these bioactive compounds has been focused on food science due to reports that the fruit would possess important bioactivities, such as being antioxidant, anti-inflammatory, and antitumoral [7]. It has also been hypothesized that the fruit could act on obesity and its related metabolic diseases, as it could help with the regulation of lipids and triglycerides in the adipocytes [10].

As there are a few commercial suppliers for the *C. sinensis* extract, in this work we studied CitrusiM® (Fagron, Brazil), an extract of *C. sinensis* (L.) Osbeck marketed as the only one with quality approved by the US Food and Drug Administration [11]. The present study aimed to infer the effects of CitruSiM® and understand its benefits for body composition. We did so under a Bayesian framework of the Rubin Causal Model, and considered possible relationships between both diet compliance and exercise level (which were intermediate outcomes in the study) and two outcomes of primary interest: percentage of lean mass and percentage of fat mass.

2. Materials and methods

2.1. Participants

One hundred and six men and women were recruited in Juiz de Fora (Minas Gerais state, Brazil) between July and September 2018. The participants' ages were between 20 and 55, their BMI were between 25 and 35 kg/m^2, and their abdominal fat by bioimpedance was greater than 20% of body weight. No participant took any drug or dietary supplement during the intervention. Pregnant women, smokers, and patients with a history of thyroid disease, cardiovascular disease, diabetes, use of weight loss medication, laxatives or diuretics, presenting recent unexplained weight loss or gain, or any other significant comorbidities were not eligible for the study.

2.2. Study design

This was a randomized, double-blind, placebo-controlled trial. Participants were randomly assigned to a three-month treatment, and each of the treatment groups corresponded to one of three different daily doses of the medicine (allocation ratio: 1:1:1). Control (group 1, n = 36) was composed of microcrystalline cellulose and burgundy food coloring (to mimic the product); Groups 2 (n = 35) and 3 (n = 35) used daily doses of 0.5 g and 1 g of CitrusiM® (Fig. 1). Sample size was determined based on availability of products to test. CitrusiM® samples were kindly donated by Fagron (São Paulo, Brazil). Participants were instructed to take the capsules with 200 mL of water at 15 h every day. All patients met with a nutritionist and were instructed to maintain a standardized diet (1575 Kcal/day) with their assigned treatments. During the study, participants were allowed to maintain their daily routine, including physical exercise.

Patients and the nutritionist were blinded throughout the whole study. A pharmacist (H.C.P.) generated the random sequence and enrolled the participants. The random allocation sequence was computer generated: a list of continuous study numbers was generated with a random allocation to treatment 1 or 2 or 3. Study numbers were consecutive and given to patients by the staff at inclusion. The nutritionist explained the trial to the patient and obtained the patient's consent, as well as conducted the clinical examination. Staff gave the treatments according to sequence number, with no mention of the groups on the label.

Fig. 1. Capsules used throughout the study (group treated with the higher dose of *Citrus sinensis 1g*).

The study protocol was approved by The Research Ethics Committee of the Faculdade de Ciências Médicas e da Saúde de Juiz de Fora, and informed consent was obtained from all participants included (approval number 2.767.611). Full protocol can be assessed at plataformabrasil.gov.br.

2.3. Clinical evaluation

Clinical evaluation was performed at baseline and after three months of treatment, and any adverse effects observed were recorded during clinic visits. Bioimpedance analysis was performed with an InBody120 (InBody Inc., Michigan, USA) balance. The intermediate outcomes of diet compliance (in percent) and amount of exercise (either none, low level, or high level) were recorded. Height were determined with measuring tape.

2.4. Statistical analysis

We aimed to infer the causal effects of CitrusiM®, and understand its benefits for weight loss. We did so under a Bayesian framework of the Rubin Causal Model [12–15], accounting for possible relationships between both diet compliance and exercise level and the two outcomes of interest. To understand the possible relationships, causal estimands for the linear and quadratic effects of the treatments were defined in terms of potential outcomes, and considered for inference. Causal inferences were obtained via the posterior distributions for the causal stamens for each outcome variable. The posterior distributions are effectively constructed by repeatedly imputing missing potential outcomes under a Bayesian regression model. Further details on this inferential approach can be found in the Supplementary Material, and are also provided by Imbens and Rubin [15]. In all of our regression models, we incorporated predictors defined in terms of the treatment indicator that explicitly capture the linear and quadratic effects.

It is important to note that diet compliance and exercise level are outcomes in their own right (because they are measured after treatment assignment), and that they could be related to our outcomes of interest. To account for possible relationships between the intermediate outcomes of diet compliance and exercise level with the final outcomes for our causal inferences, we specified a model in which the former two outcomes were first modeled as a function of the treatment and covariates, and then the final outcomes were modeled as a function of the treatment, covariates, and intermediate outcomes. Specifically, we first modeled exercise levels as a function of treatment using a Multinomial model, then modeled the logistic transformed diet compliances as a function of the treatment contrasts, covariates, and exercise levels, and finally modeled a response variable Y of interest as a function of the treatment contrasts, covariates, exercise level, and diet compliance. We then imputed all missing potential outcomes under the Bayesian regression model that corresponded to this specification, and thereby accounted for the relationships between the intermediate and final outcomes to perform causal inferences on the primary estimands of interest. A Multinomial logistic regression model for exercise level incorporating a subject's covariates was not considered here due to the small sample size. The mathematical formulations of the regression models are provided in the Supplementary Information.

3. Results and discussion

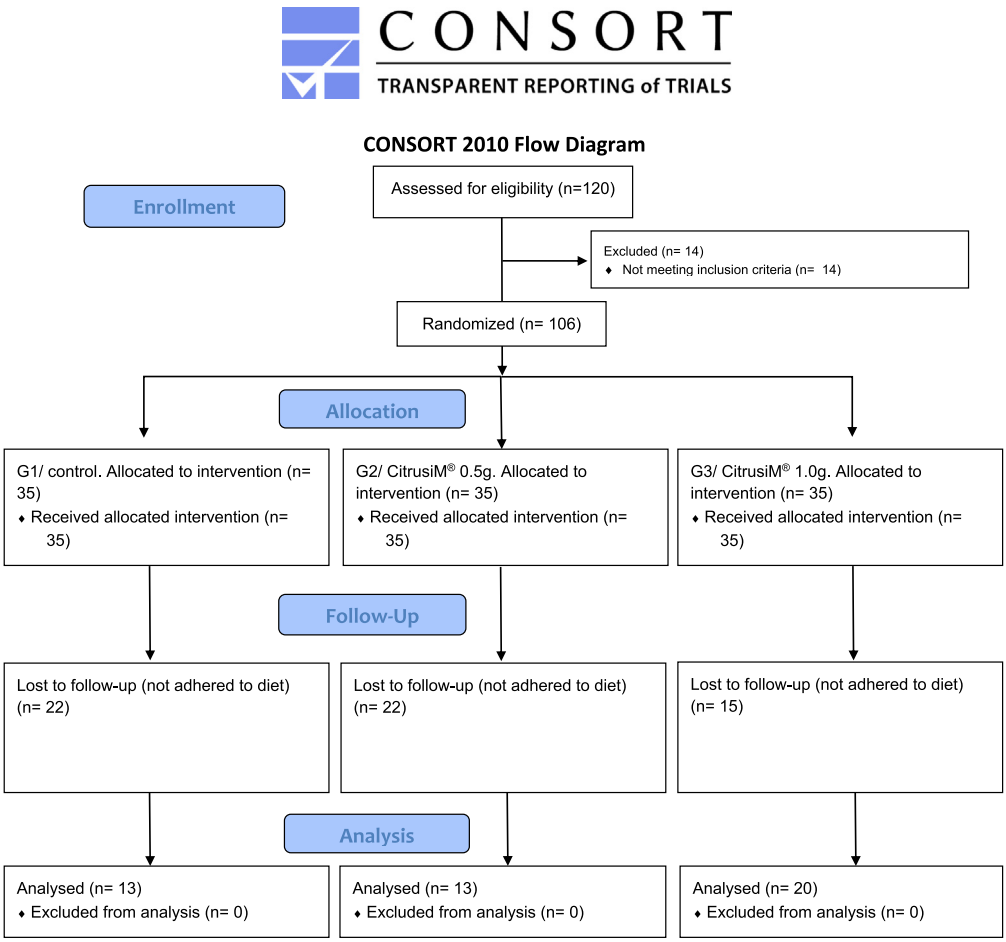
A total of 106 participants were originally enrolled in the double-blind experiment through the whole year of 2018 and 46 finished the study (Flow Diagram 1). Of these 46 individuals, 13 were from control, 13 had 0.5 g, and 20 had 1 g of CitrusiM®. Therefore, limitation of this trial was the small sample size.

Also, 80% of these subjects were female. In regard to the exercise levels among the participants, 15 did not exercise, 12 had a low level, and 19 had a high level. Table 1 summarizes the covariates.

Table 1
Descriptive statistics for the subjects' characteristics.

Covariate	Minimum	Maximum	Median	Mean	Standard deviation	Missing values
Age	21	54	37.50	37.28	11.28	10
Height (cm)	150	186	162.5	163.9	7.80	0
Initial Weight (kg)	58.80	117.20	78.35	80.03	13.55	0
Initial BMI	23	42.20	29.25	29.78	4.42	0
Initial Lean Mass (%)	27.90	44.94	34.40	35.04	4.45	0
Initial Fat Mass (%)	21.30	50.20	37.05	36.88	7.17	0

BMI = body mass index.



At the end of the three-month period of treatment, we could see that there was a decrease in fat mass and waist circumference for the groups treated, compared to the control (Fig. 2 and Table 2), as well an increase in lean mass for the same groups. These results are already weighted for diet and exercise compliance. Detailed results can be found in Supplementary Material.

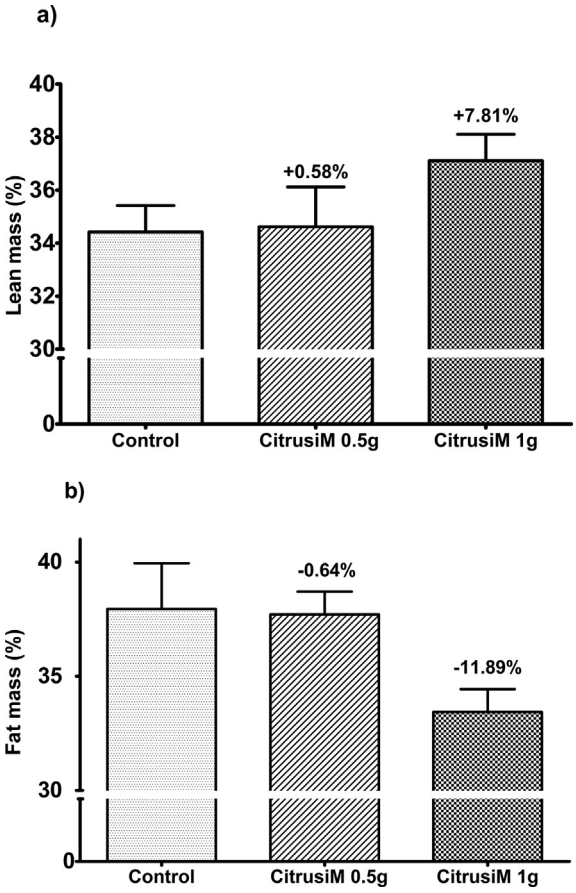


Fig. 2. Results on body composition parameter after three months of treatment with *Citrus sinensis*.

There is currently a growing scientific interest in nutraceuticals because of their potential effects on the treatment of obesity and being overweight [16]. In the present study, we observed that CitrusiM® could increase lean body mass, and reduce fat mass. These effects agree with a theoretical rationale for the material, as it contains a powerful set of biactive molecules, including flavonoids, carotenoids, and anthocyanins in high concentrations. One particular molecule in high concentration is cyanidin-3-glucoside, which seems to be associated with reductions in body weight and fat accumulation [17].

Table 2
Bayesian inferences on the causal effects of CitrusiM® on each outcome.

Outcome	Causal effect	Posterior mean	90% credible interval	95% credible interval
Total body weight	Linear	−2.29	(− 5.32, 0.66)	(− 5.99, 1.31)
	Quadratic	−0.33	(− 2.96, 2.25)	(− 3.70, 2.75)
Lean Mass	Linear	1.18	(0.27, 2.06)	(0.10, 2.19)
	Quadratic	0.27	(− 0.42, 1.04)	(− 0.69, 1.11)
Fat Mass	Linear	−1.55	(− 2.94, − 0.14)	(− 3.23, 0.12)
	Quadratic	−0.68	(− 1.87, 0.51)	(− 2.11, 0.8)

Previous studies conducted with the dried extract of *C. sinensis* demonstrated similar results. The study of Cardile et al. [17], for example, was carried out with treatments of 400 mg of *C. sinensis* once each day for 30 healthy overweight volunteers during a 12-week treatment period. They found no significant weight change, which agrees with our findings. Kaneko et al. [18], also evaluated the same product in the same dosage as Cardile et al. They performed a stratified analysis to verify the effects of the product in non-obese patients ($n = 4$ for treated; and $n = 2$ for placebo). The authors affirmed that they verified the same positive effects as the ones found by our group - however, this was a very small study. Thus, the combination of these two studies with our study enables the hypothesis that Moro orange extract yields more effective results when used in overweight patients compared to obese ones.

Although our study was conducted with a larger sample size than the other studies, our sample size could have been larger if patients were not loss during the course of the study due to drop out (which arose because of the difficulty in adhering to the 1575 Kcal/day diet). Even with no side effects observed on the participants, given the current sample size and the results obtained we recommend further validation experiments with a larger number of subjects to confirm the results described in this report.

4. Conclusions

CitrusiM® appears to have a significant effect on fat mass reduction, as well as a lean mass increase, for both doses used (0.5 or 1 g, daily). However, there exists statistical evidence of the existence of an optimum dose (1 g/day) for reducing waist circumference.

Conflicts of interest

The authors declare no conflict of interest nor funding. Samples were kindly donated by Fagron (São Paulo, Brazil) upon request.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.yclnex.2019.08.002

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