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A study protocol for a randomized clinical trial on exposure and effects of pesticides consumption - the PEST-EXPO Brazil study

Júlia Oliveira Penteado[a,*], Laiz Coutelle Honscha[a], Caroline Lopes Feijo Fernandes[a], Paula Florencio Ramires[a], Ronan Adler Tavella[a], Fernanda Oliveira Reis[a], Mariane Pergher Soares[a], Lisiane Martins Volcão[b], Marina dos Santos[c], Ana Luiza Muccillo-Baisch[a], Flavio Manoel Rodrigues da Silva-Júnior[a]

[a] *Federal University of Rio Grande, Itália Avenue, Km 8, Carreiros, 96203-900 Rio Grande, Rio Grande do Sul, Brazil*
[b] *Southern Frontier University, Jacob Reinaldo Haupenthal Avenue, 1580, 9790-000 Cerro Largo, RS, Brazil*
[c] *Federal University of Pampa, Luiz Joaquim de Sá Brito Street, s/n, 97650-000 Itaqui, RS, Brazil*

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ABSTRACT

Randomized clinical trials are considered the gold standard for studies with dietary interventions, which is mainly due to the fact that they can establish causal relationships between food exposure and body composition measures or biomarkers. The aim of this study was to describe the details of a double-blind, randomized, clinical trial protocol to identify, characterize and evaluate the effects of human dietary exposure to pesticide residues in food. Specific aspects of planning (development of a research question, determination of objectives, selection of participants, randomization and blinding) and performance (recruitment of participants, measures to improve adherence, data collection, follow-up and evaluation of results) are addressed in this study. The study design proved effective in characterizing dietary patterns with foods originating from both conventional and organic agriculture. A total of 148 individuals were recruited for the study. The conventional group was represented by 47 % of the sample and the organic group was represented by 53 %. The practice of evidence-based nutrition has demanded that trials be well designed and systematically performed in the field of clinical nutrition. Therefore, this clinical trial emphasizes the importance of improving studies with toxicological nutrition that assess sources of exposure through food.

- This double-blind, randomized clinical trial details the protocol for identifying, characterizing, and evaluating the effects of dietary exposure to pesticide residues.
- The protocol demonstrates that well-designed and systematically conducted trials emphasize the importance of robust methodologies in evidence-based nutrition.
- In the face of the global climate crisis, this clinical trial underscores the importance of enhancing studies in toxicological nutrition, particularly those evaluating sources of exposure through food, to better understand the dietary impacts on health.

Specifications table

Subject area:	Food Science
More specific subject area:	Toxicological clinical nutrition
Name of your protocol:	PEST-EXPO Brazil study

(*continued on next page*)

* Corresponding author.
E-mail address: icb@furg.br (J.O. Penteado).

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Reagents/tools:	Universal collector and falcon (urine test), tube and eppendorf were used for blood collection.
Experimental design:	This was a randomized double-blind clinical trial parallel with a 14-day intervention performed at Universidade Federal do Rio Grande and is part of the doctoral thesis deposited at https://sistemas.furg.br/sistemas/sab/arquivos/bdtd/0000015027.pdf. The 148 individuals in the RCT were randomly assigned to two experimental groups: conventional group and organic group. The daily diet for the RCT was planned in collaboration with the University's team of nutritionists. The menu and quantity of food offered to both groups (conventional and organic) were identical. For breakfast, participants were offered coffee or milk, bread with cold cuts, and a portion of fruits. Snacks such as biscuits or cakes were provided for the afternoon break. At lunch and dinner, the meal was composed of vegetables (raw and cooked), portions of food from the group of cereals and tubers, portions of legumes and portions of meat, as well as 2 eggs or vegetable protein. On the first day of intervention and on the fifteenth day, before the first meal, urine and blood samples were collected for the analysis of RCT outcomes. A total of 113 pesticides were analyzed in urine, while biochemical and genetic parameters were assessed in the blood.
Trial registration:	The RCT was registered under the number n° RBR-8d85hs4 in Brazilian Registry of Clinical Trials, available at: https://ensaiosclinicos.gov.br/rg/RBR-8d85hs4, entitled: Evaluation of the health effects of food consumption with agrochemical residues.
Ethics:	The study protocol was approved by the Health Research Ethics Committee of the Universidade Federal do Rio Grande - FURG and approved with the registry number of 55/2019. All of the participants were given the Free and Informed Consent Form.
Value of the Protocol:	1) The developed protocol sets a gold standard for assessing causal relationships between dietary exposure to foods with pesticide residues and their impacts on health. 2) Studies like the one implemented in this protocol are essential for monitoring exposure to environmental contaminants, aiming to ensure Food and Nutritional Security.

Description of protocol

Diet can be a source of exposure to hazardous environmental chemicals through food contaminated by residues of substances during food production or processing and in marine and agricultural food chains [1]. The quality of food has been impacted by the use of pesticides in plantations for pest control [2,3]. Pesticides have been identified in various food groups, particularly in fruits and vegetables [4,5]. It is noteworthy that pesticide residues have also been detected in processed foods [Anon., 6], potentially interfering with the nutritional security of the population [7].

Pesticide exposure via food consumption has been associated with several adverse health effects, including decreased cognitive scores and food allergies in children [8,9], the development of asthma [10], obesity [11] and cancer [12], as well as negative impacts on the reproductive system [13] and fetal development [14,15]. In addition, biomonitoring studies have indicated that pesticide levels and their metabolites are higher in individuals with a diet based on conventionally produced foods when compared to organic items [4,16–25] (Table S1). The replacement of only conventional fruits and vegetables with organic items was sufficient to reduce urinary levels of pesticides to undetectable levels [25].

Organic products market has grown rapidly in recent decades [Anon., 26]. In general, organic foods do not allow for the use of synthetic fertilizers, pesticides or genetically modified organisms and restrict the use of veterinary drugs. Consequently, organic products are less likely to contain pesticide residues than those grown through conventional production methods. Observational studies by Vigar et al. [27] have shown positive health outcomes related to the consumption of organic foods, but they underscore the urgent need for long-term clinical trials involving the total replacement of the diet with certified organic foods.

The randomized clinical trial (RCT) is the experimental design considered to be the gold standard to assess efficacy in clinical research and constitutes evidence for health treatment [28], as well as reducing the confusion and biases observed in experimental studies [29]. However, RCTs with partial food replacement performed in a short period of time or with a small sample size may not significantly reflect the effects of diet. Moreover, those RCTs with total replacement from conventional to organic diets (4 to 22 days) observed health benefits and a significant reduction in pesticide metabolites excreted in urine [30–35] (Table S2).

Additional studies are required, particularly those addressing long-term exposure and whole-diet replacement RCTs employing certified organic interventions to provide reliable scientific evidence regarding the measurable health benefits of an organic diet. We describe details of a double-blind randomized clinical trial protocol to identify, characterize and evaluate the effects of human dietary exposure to pesticide residues in food. Specific objectives about aspects of planning (development of a research question, determination of objectives, selection of participants, randomization and blinding) and performance (recruitment of participants, measures to improve adherence, data collection, follow-up and evaluation of results) are addressed in this study.

Trial design

This was a randomized double-blind clinical trial parallel with a 14-day intervention performed at Universidade Federal do Rio Grande (32°04′18.26″S and 52°09′59.33″W). The intervention was preceded by a seven-day washout period, in which participants received the same conventional diet. The study schedule for enrollment, interventions and assessments is summarized in Table 1. All of the participants were given the Free and Informed Consent Form. The study in accordance with the ethical standards proposed by Resolution 466/12 of the National Health Council of the Ministry of Health, which regulates Brazilian research involving human beings. The study protocol was approved by the Health Research Ethics Committee of the Universidade Federal do Rio Grande - FURG with the registry number of 55/2019. The RCT was registered under the number n° RBR-8d85hs4 in Brazilian Registry of Clinical

Table 1
Summary of study schedule for enrollment, intervention, and evaluation.

Interventions	Period				
	Pré-RCT	Pilot study 1–3 days	Washout 4–10 days	RCT 11–24 days	Post RCT 25 days
Recruitment Strategy	√				
Screening and enrollment	√				
Consent Term with the Study	√				
Organization and delivery of meals with the RCT team		√			
Standardization of participants' diet			√		
Sample randomization (allocation ratio: randomized with exposed and unexposed group)			√		
Data collection				√	
Intervention				√	
Data collection					√

Trials, available at: https://ensaiosclinicos.gov.br/rg/RBR-8d85hs4, entitled: Evaluation of the health effects of food consumption with agrochemical residues. The study was carried out in accordance with the CONSORT (Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomized trials), and in conformity with WHO standards.

Sample

The participants included students residing in university housing at the Universidade Federal do Rio Grande. Eligibility Flowchart of RCT Participantsis described in Fig. 1.

Pre-RCT

Recruitment strategy

The research was presented to the Dean of Student Affairs and the university nutritionists. After the consent of these managers, a meeting was scheduled with all of the students representing the university houses offered by the university on its campus to invite and publicize the study.

The students were invited to participate in the study by using posters and advertisements on the university's website and radio. Banners, pamphlets and plates with a brief explanation of the research and researchers' contact information for questions were disclosed in the university social environments, such as cafeterias, coffee shops and coexistence centers on the campus. Furthermore, the study was published in classrooms, and students were invited by the researchers to learn about the study.

The meetings were held in the social environments of the university houses. In the meetings, the benefits of the research, the scientific impacts related to the theme of the study, the clarification of the eligibility criteria and restrictions during the study period were discussed. In addition, the possible risks and the appropriate measures that the researchers would take in case of any discomfort or risks to the participant's health were also explained. Students who were interested in participating in the study provided their data to the researchers, including full name, date of birth, email address and telephone number.

Inclusion and exclusion criteria

The study eligibility criteria included the following: 1) men and women; 2) age between 18 and 40 years; and 3) healthy individuals without chronic noncommunicable diseases or autoimmune diseases. Women who were pregnant or breastfeeding were excluded from the study.

Training for data collection

All of the research teams received two trainings before the start of the study. First, they received a training of Standards of Good Hygiene Practices at meal production and how to use Personal Protective Equipment, as administered by a competent and registered professional. Another training concerned RCTs, which highlighted the importance of complying with the proposed experimental design and respecting the rules of randomization and blinding.

Outcomes

Following the completion of the study, the primary outcomes related to the development of the ECR were expressed in absolute and relative frequency for sample characterization. Specifically, this encompassed variables such as gender, age, place of residence, dietary type, and the profile of food intolerances and allergies, and anthropometric measurements. The menu offered during the 14 days of intervention is arranged in the format of a table, described by days of the week and food groups (cereals, legumes, meat,

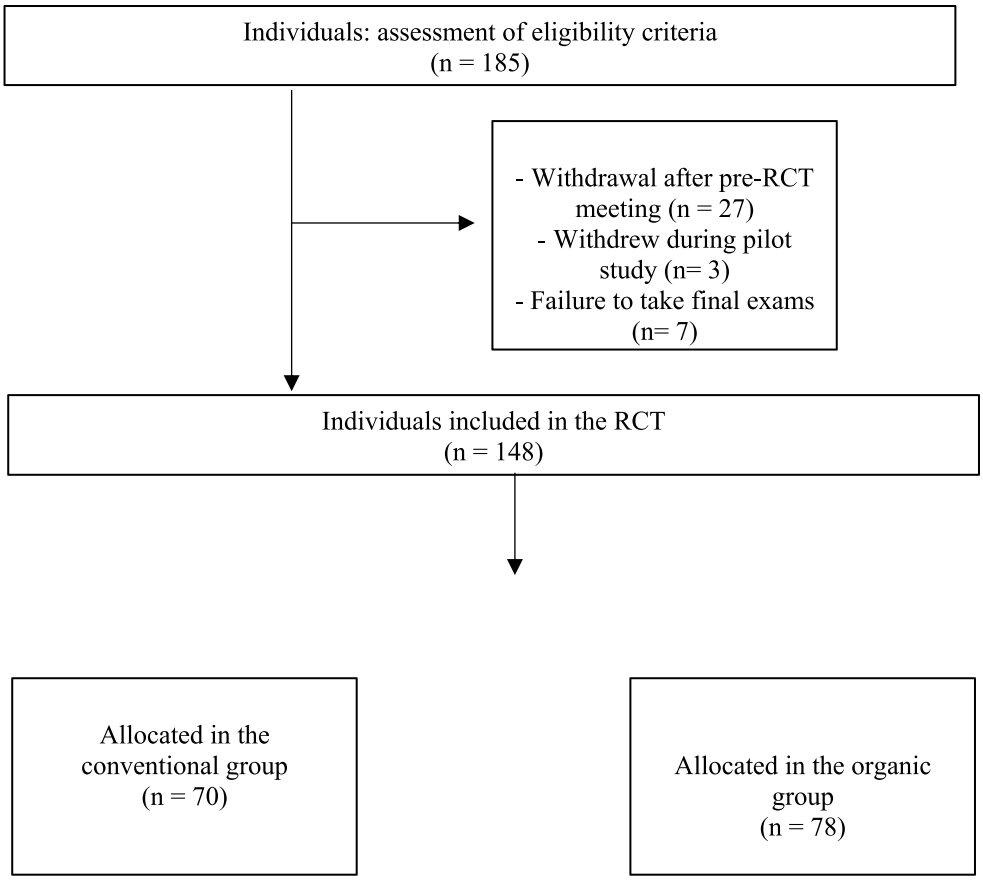

Fig. 1. Eligibility Flowchart of RCT Participants.

eggs and vegetarian portion, raw and cooked salads and fruits).Finally, the pesticides measured in the urine of the participants were described in a Table S3, with all the specifications: retention time, transition – quantification, transition - confirmation.

The secondary outcomes pertain to the logistical aspects of conducting the examinations, encompassing the professionals responsible for specimen collection, the site, the employed equipment, and pre-instructional guidance. Adherence tools to the ECR were also detailed and provided as Supplementary Material 5. Pre-specified adverse effects were descriptively measured by the participants themselves and presented in Table with absolute and relative frequencies (Table 3).

Sample size

The sample size was calculated using a repeated measures design formula considering 2 factors: group and exhitibion. This study set the power size to 80 % ($\alpha = 0.05$) in 2 groups using OpenEpi software - open source epidemiological statistics for public health, resulting in a total of 150 participants. We selected participants with an extra total, considering the 10 % dropouts.

Clinical trial

Pilot study and washout period

A three-day pilot study was performed with researchers, cooks and participants to allow them adapt to the RCT methodology. At this point in time, the researchers adjusted the logistical details to the day of the intervention. In addition, information on the training was reinforced to the team of cooks who were responsible for preparing the study meals. During the washout period of seven consecutive days, all of the participants went through a baseline period, in which no organic or pesticide-free food was offered by the researchers. After this time period, the participants were randomized and allocated to the experimental groups, including the conventional (unexposed) and organic (exposed) groups.

Randomization of participants e blinding

Simple randomization was performed in this study. The names of all of the volunteers who had previously signed the Free and Informed Consent Form were added into an Excel spreadsheet in alphabetical order and numbered from 1 to 148, which represented the total number of clinical trial volunteers. Afterward, the Excel random function was chosen for 0 and 1, wherein 0 was the

conventional group, and 1 was the organic group. The randomization process was performed by a researcher not involved in the RCT. Participants were assigned respective identification numbers and exclusively utilized these numbers throughout the study's duration. Similarly, the researchers engaged in preparation, distribution, examination execution, and result analysis had access to a list correlating to the conventional and organic groups, devoid of personal identification.

Food products

The university provided all of the conventional food, whereas organic food, such as processed food (bread and cookies) and food of animal origin (meat, eggs and milk), was provided by Korin and other organic food companies with a certification seal, and organic fruits and vegetables were obtained on a daily basis by local farmers with an organic certification seal. All of the food that was used to prepare the meals was stored in the kitchen at the university restaurant. The other foods offered for daily consumption, such as yerba mate, popcorn, cookies and cakes, were repackaged on a daily basis in brown paper envelopes and identified on the outside with the participant's randomization number.

Diet

The daily diet of the RCT was planned with the university's team of nutritionists and is presented in Table S3. The menu and the amount of food offered in the diets for both groups (conventional and organic) were identical. For breakfast, coffee or milk, bread with cold cuts and a portion of fruits were offered, after which cookies or cakes were delivered for the afternoon snack. At lunch and dinner, the meal was composed of vegetables (raw and cooked), portions of food from the group of cereals and tubers, portions of legumes and portions of meat, as well as 2 eggs or vegetable protein. The distribution of kilocalories from Total Energy Value (TEV) for each meal was 20–25 % for breakfast, 30–40 % for lunch, 10–15 % for afternoon snack and 30–40 % for dinner, supplemented with a piece of fruit. The daily intake per meal of fats, carbohydrates and proteins for each meal respected the reference food intake [Anon., 36]. Individual portions were delivered to participants according to estimated energy consumption. Participants were encouraged not to consume foods and beverages other than those provided by the researchers. In addition, a food diary was provided to describe daily fluid intake and any extra food and drink that had been consumed.

Meal preparation

All of the main meals (lunch and dinner) were prepared in the restaurant's kitchen by the university staff. Organic food was also prepared inside of the kitchen (but in a separate sector by a specific cook who was responsible for organic meals). In addition, the prepreparation, preparation and cooking utensils (pots, cutlery, boards and vats) of organic food were sanitized and stored separately from the others. After the cooking stage, the food was placed in the RCT buffet located inside of the kitchen. This buffet was divided in a way that included conventional foods on one side and organic foods on the other (both with the same food groups). Breakfast and afternoon snacks were delivered at the same time during breakfast and inside of the cafeteria of the university's restaurant.

Meal delivery

The meals were offered at the times that were usually stipulated by the university: breakfast at 7 h to 9 h, lunch at 11 h to 14 h and dinner at 18 h to 22 h. Study participants identified themselves at the restaurant door with their randomization number through the identification provided by the researchers and were oriented by researcher 1 to the kitchen door. At this moment, researchers 2 and 3 communicated to researchers 4, 5, 6, 7 and 8 inside of the kitchen as to the number of requested dishes. Those individuals in charge prepared the dishes inside of the kitchen and handed it to researchers 2 and 3, and the participants received the meals in the prepared dishes (Fig. 2).

Exams

To perform the exams, a previously sanitized university building was provided, with all of the necessary equipment for the collections. Blood and urine were collected, and an anthropometric assessment was performed on the participants on the first day of the RCT (before the first meal) and on the fifteenth day after the intervention period (i.e., on the initial day and on the last day of the intervention). Participants were instructed to fast for at least 8 h, to not consume alcoholic beverages and to abstain from physical exercise in the 24 h prior to the collection of the exams.

Blood and urine collection

Blood collection was performed by researchers with training in nursing and biomedicine, and urine was collected by the participants themselves in sterile plastic containers and delivered to the researchers.

Blood samples were collected and immediately centrifuged for 10 min at 3000 rpm in a Sigma centrifuge to obtain the plasma, after which they were taken to an ultrafreezer at −80 ˚C until the time of analysis. Liver indicators (glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase) and protein indicators (urea and creatinine) were evaluated by using Labtest kits, and genetic damage was evaluated via the comet assay. The comet assay was performed immediately after the collection of the blood samples.

Urine samples were transferred from the universal collector to Falcon tubes and stored at −80 ˚C in ultrafreezers. Samples were analyzed for pesticide biomarkers and metabolites according to the method of Arias et al. [37]. Five grams of sample was weighed in a 15 ml polypropylene tube; subsequently, 1 ml of acetonitrile was added to the mixture, which was vortexed for 1 min. Afterwards, 2 g of $MgSO_4$ was added to the mixture, which was mixed again for 3 min, followed by centrifugation for 5 min at 7000 rpm (7793 × *g*). Finally, 500 μL of the acetonitrile layer was collected and diluted to 1 mL with ultrapure water in an HPLC vial, and 20 μL was

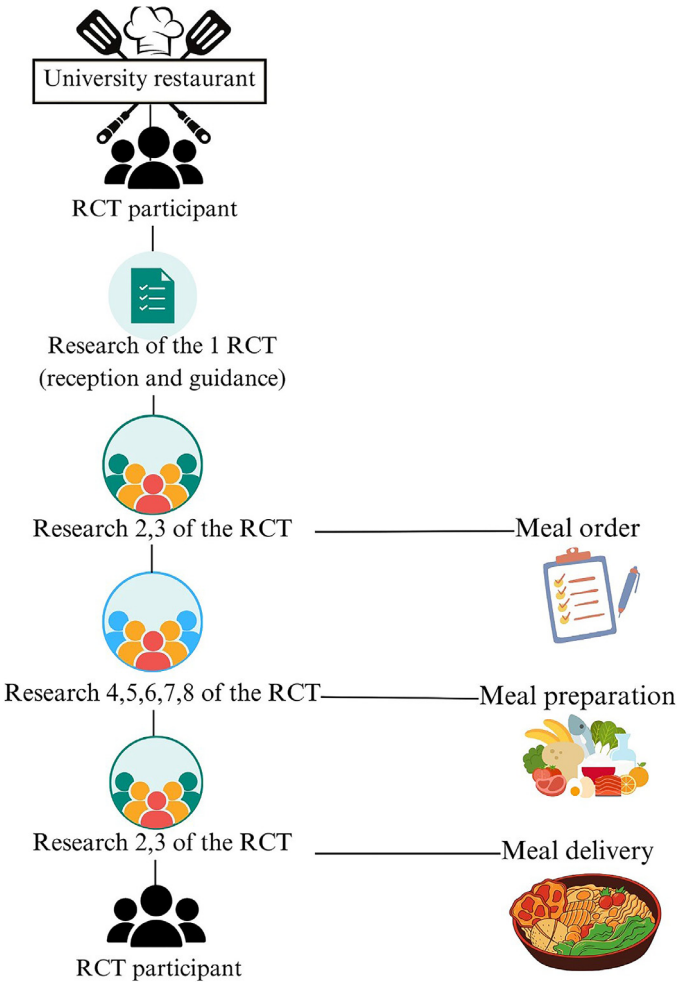

Fig. 2. Meal delivery flowchart for participants.

injected into the chromatographic system. The experiments were performed in triplicate. The pesticides and metabolites that were analyzed are described in Supplementary Table S4.

Dietary intake and health aspects

A food diary was prepared to quantify additional foods that were ingested by the participants during the intervention period of the study. The first page of the instrument contained guidelines that expressed the importance of describing the types of ingested food, the amount in household measures and the brand that was consumed. They were also instructed to describe the amount of water that was ingested for each day of the intervention. To make it easier to fill in the diary, examples of utensils and foods were added to determine quantity and portion size (Supplementary Material 5). To assess adverse health effects during the RCT, we added a signs and symptoms questionnaire at the end of the Food Diary.

Mechanisms for maintaining adherence to the RCT

Application (APP)

In order to enhance participant engagement in the study, a complimentary mobile application was designed and developed. This app featured comprehensive information regarding the RCT, pesticides and their impact on health, as well as the full menu for each day of the intervention. Additionally, it included functionalities such as the ability to capture photographs of daily meals, a notepad for personal notes, and a calendar displaying examination dates and scheduled activities or games.

Table 2
Social characteristics and type of diet of individuals in the Clinical Trial sample, Rio Grande, Brazil (n = 148).

Variables	Conventional group (n = 70) n (%)	Organic group (n = 78) n (%)	Total n (%)	p-value
Sex				
Masculine	30 (20)	39 (26.5)	69 (46.5)	p = 0.241
Feminine	40 (27)	39 (26.5)	79 (53.5)	
Age				
≤ 20	24 (16.2)	19 (12.8)	43 (29)	p = 0.098
21 – 29	41 (27.7)	56 (37.8)	97 (65,5)	
≥ 30 anos	6 (4.1)	2 (1.4)	8 (5.5)	
Home				
University housing	62 (41.9)	75 (50.7)	137 (92.6)	p = 0.074
Own house	8 (5.4)	3 (2)	11 (7.4)	
Type of Diet				
Ominivorous	61 (41.2)	69 (46.5)	130 (87.7)	p = 0.497
Vegetarian	5 (3.4)	8 (5.4)	13 (8.8)	
Vegan	2 (1.4)	0 (0)	2 (1.4)	
Lacto-ovo-vegetarian	2 (1.4)	1 (0.7)	3 (2.1)	
Food allergies or intolerances	4 (2.7)	1 (0.7)	5 (3.4)	p = 0.833
Lactose	1 (0.7)	0 (0)	1 (0.7)	
Others	65 (43.9)	77 (52)	142 (95.9)	
No allergies or intolerances				

Fisher's exact test ($p \leq 0.05$).

Games

The initiative to arrange and promote weekend games as a component of the RCT was undertaken by the university student sports association. Exclusive sports tournaments were organized for the participants during these weekends.

Date analysis

The sample characterization data were subjected to analysis of assumptions, wherein normality was evaluated by using the Shapiro Wilk test; additionally, homoscedasticity was evaluated by using the Hartley test, and the independence of residues was assessed via graphical analysis. As they did not fit the normality distribution, the data were then analyzed in a descriptive manner. The variables were quantitatively analyzed by using IBM SPSS Statistics software, version 28.0.0.0, wherein descriptive statistics were calculated, such as absolute (n) and relative (%) frequencies. Moreover, comparisons between the conventional and organic groups were assessed by using Fisher's exact test for the following variables: age, sex, type of diet, household and food allergies.

Protocol validation

In this study, we developed a double-blind RCT was registered under the number n° RBR-8d85hs4. Participants were recruited between June and July 2019 and the study was carried out between August and September 2019. Of the 185 individuals who expressed interest in participating in the study, 27 volunteers dropped out after the pre-RCT meeting. Of the total of 158 participants who received the instructions and signed the Free and Informed Consent Form, 148 individuals completed the study. Three individuals dropped out during the pilot study, and seven individuals did not attend the final exam collection, totaling 6 % of losses or dropouts (Fig. 1).

In the realm of human nutrition, Randomized Controlled Trials (RCTs) are widely acknowledged as the gold standard for establishing causal relationships between exposure to nutrients, foods, or dietary patterns and predefined outcome measures, such as biomarkers [38]. In this study, participants were randomly assigned to either the conventional or organic experimental groups.

In an experimental condition (EC), the implementation of randomization is crucial to counteract the selection bias among participants included in the study. Differences in individual characteristics can act as confounding factors, potentially making it challenging or even impossible to ascertain the true impact of the intervention [29]. Moreover, the incorporation of a double-blind design ensures that any care delivered during the study, whether by the researcher or the participant, remains uninfluenced, thereby minimizing biases [27].

The characteristics of the participants are shown in Table 2. The conventional group was represented by 47 % of the sample (30 men and 40 women), and the organic group was represented by 53 % (39 men and 39 women). The age ranged between 23 and 27 years, and 92.6 % of the participants lived in the accommodations offered by the university. Individuals who had any food allergy/intolerance (lactose, n = 5, basil allergy, n = 1) or dietary restrictions due to personal taste received meals that were suited to their preferences.

The menu was devised for the 14-day intervention, encompassing various groups of grains and tubers: rice, pasta, potatoes, and cassava. Legumes such as beans and chickpeas, along with protein sources of both animal and plant origin, were included to

Table 3
Adverse effects mentioned during the Clinical Trial period ($N = 148$).

Adverse effects	Conventional group ($N = 70$) n (%)	Organic group ($N = 78$) n (%)
Dizziness	2 (2.9)	2 (2.6)
Headache	5 (7.1)	4 (5.1)
Nausea	2 (2.9)	2 (2.6)
Vomit	5 (7.1)	–
Motion scikness	3 (4.3)	4 (5.1)
Reflux	2 (2.9)	2 (2.6)
Abdominal pain	1 (1.4)	5 (6.4)
Diarrhea	6 (8.6)	2 (2.6)

accommodate individuals with vegan or vegetarian diets. Furthermore, two eggs were available to all participants who desired them. Raw and cooked fruits, as well as salads, were provided in portions to participants during all meals (Table S3).

However, conducting double-blind RCTs for food-based treatments poses challenges. In contrast to pharmaceutical trials, RCTs in human nutrition frequently involve testing foods with distinct taste, appearance, texture, and/or smell, making it challenging to maintain blinding [39]. To address this issue, we ensured both groups received food of the same quality, with the only discernible difference being the cultivation method (conventional or organic). It's worth noting that other RCTs have utilized single blinding [40,41], while some have successfully implemented double-blinding, similar to the approach outlined in this study [30,42].

Participants' adherence to meal consumption was similar between the groups. The frequency at lunch meals was 88 %, the frequency at dinner was 78 % and decreased adherence was observed at breakfast at 57 %. All of the participants received snacks for the rest of the day, including those who did not eat breakfast. In addition, all of the participants received extra foods, such as popcorn or yerba mate.

To minimize adherence bias, we recruited a large sample ($n = 148$) compared to other RCTs (Table S2). The most effective recruitment was through the use of targeted correspondence (emails); however, it is necessary to highlight the need to combine recruitment methods (emails, printed and digital leaflets, etc.) [43]. With sufficiently large samples, randomization can balance all of the unknown factors related to the participant and eliminate possible biases that can be generated by confounding factors [28].

Nutritional research encounters distinctive challenges. Altering an entire diet has the potential to induce compensatory changes in its composition, thereby affecting the results and interpretations of the findings [44]. To mitigate the impact of changes in diet composition and prevent compensatory behaviors in total or relative intake, adherence measures to the randomized clinical trial (RCT) were instituted. All participants maintained a comprehensive food diary, documenting all additional foods consumed during the study.

Regarding adverse health effects that were reported during the RCT, 17.5 % of participants in the conventional group reported diarrhea as the most prevalent effect. In the organic group, 14 % of the participants reported of being sick at some point during the study (Table 3). In the conventional group, one person reported of having flu-like symptoms; moreover, in the organic group, three participants reported of the same symptoms. The most commonly used medicines during the study period were analgesics (Conventional = 8, Organic = 14) and antipyretics (Conventional = 6, Organic = 14). All of the study participants completed the adverse effects questionnaire and the Food Diary.

The study examinations were performed before and after the dietary intervention. The trained multidisciplinary team performed the collections in an identified way according to randomization. Nutrition professionals received standardized forms to identify the participant by randomization number and all of the parameters to be evaluated.

Moreover, blood and urine samples were carefully stored for processing and storage temperature, light exposure and aliquot volumes for each sample. Sample labeling schemes were readable without ambiguous information or personal identification. Furthermore, participants who only attended the initial collection had their samples stored separately from the rest of the group until the time of analysis.

The collection methods used for the present study carried risks (including urine and blood collection). The collection of biological samples took place in a properly equipped, sanitized, and prepared room at the Federal University of Rio Grande. Blood sample collection was carried out by qualified professionals using appropriate materials, while urine collection was performed by the participants themselves and promptly stored by a duly trained researcher, adhering to the Ministry of Health regulations.

Collection procedures were executed with a commitment to preserving the physical and psychological integrity of participants, adhering to ethical and safety standards set for this process. Participants were duly informed that, despite all precautions in place, if any discomfort were to occur, they would be promptly referred to the nearest healthcare facility where the collection was being conducted.

Participants were assured the opportunity for clarification of doubts and access to research findings, with the understanding that they had the freedom to withdraw from the informed consent process at any point without facing any adverse consequences. Importantly, it should be highlighted that there were no associated expenses or personal compensations for individuals participating in the research.

However, common barriers to RCT performance have been widely recognized and discussed. In 2008, Duley et al. [45] identified the main difficulties in performing RCTs, including inadequate funding, complex regulations that sometimes make it difficult to

interpret results, monitoring and lack of training and understanding of the methodology. Furthermore, due to the fact that RCTs are highly controlled for both the study environment and the inclusion and exclusion criteria, this scenario generates a homogeneous population of participants and limits the generalization of results to other populations [46].

From a public health perspective, nutrition is vital to the health, economic development and lifelong productivity of the population. Therefore, an RCT is necessary for the obtained results to contribute to nutritional security and the health of the population. Specifically, if we understand that nutrition is a therapy and can prevent and promote health, there is a need for an increase in large-scale multicenter RCTs with robust methodologies, transparent reporting and the development of international research networks to advance the field. This will help to acquire, interpret and apply the best possible evidence on the effects of consuming foods produced in the conventional system with pesticides.

The present study had some limitations. Although the sample size was large enough to provide adequate statistical power, it was relatively small for population studies. Although we did our best to control the food intake of the participants, this scenario was difficult because they had free choice outside of meal times. Routine interference and eventual underreporting are possible method biases. In addition, pesticide exposure was only assessed for seasonal food groups (fruits and vegetables), as organic foods were purchased from local producers and are grown at preestablished periods. Therefore, the observed exposure only includes the foods related to the specific time period of the study. Our study methodology also does not measure the effects of the generation of pesticide metabolites that may occur naturally from environmental exposure.

The design that was performed in this study confirmed that RCT is the gold standard methodology for trials in the area of nutrition, which requires previously structured planning of the study design with transparent reports and still needs more financial support from government and private companies. The rigor of scientific research is essential to provide quality inputs on which to base the development of nutritional policies and guidelines. Failure to comply with methodological and ethical standards may compromise the scientific integrity of the study results. Thus, improving the quality of clinical research in toxicological nutrition becomes necessary to clarify the relationship between diet and exposure to contaminants for the population and to determine their health effects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Júlia Oliveira Penteado: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Laiz Coutelle Honscha:** Methodology, Writing – review & editing. **Caroline Lopes Feijo Fernandes:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Paula Florencio Ramires:** Investigation, Methodology, Writing – review & editing. **Ronan Adler Tavella:** Investigation, Methodology. **Fernanda Oliveira Reis:** Investigation, Methodology. **Mariane Pergher Soares:** Investigation, Methodology. **Lisiane Martins Volcão:** Investigation, Methodology, Writing – review & editing. **Marina dos Santos:** Investigation, Methodology. **Ana Luiza Muccillo-Baisch:** Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing. **Flavio Manoel Rodrigues da Silva-Júnior:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2024.102942.

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