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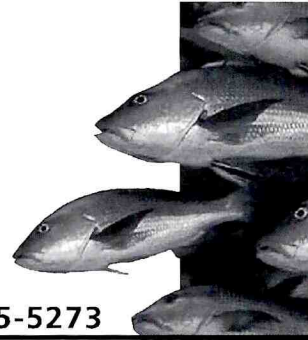
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Breastfeeding-Friendly Primary Care Initiative: Degree of implementation in a Brazilian metropolis¹

Iniciativa Unidade Básica Amiga da Amamentação: avaliação do nível de sua implantação em uma metrópole brasileira

Rosane Valéria Viana Fonseca RITO²

Inês Rugani Ribeiro de CASTRO³

Alexandre José Baptista TRAJANO⁴

Maria Auxiliadora de Souza Mendes GOMES⁵

Regina Tomie Ivata BERNAL⁶

ABSTRACT

Objective

This study assessed the degree of implementation of the Breastfeeding-Friendly Primary Care Initiative in a Brazilian metropolis.

Methods

A tool with 55 items and a maximum score of 10 points, based on validated protocols, was developed for assessing the degree of implementation of the abovementioned initiative. This tool was used on a probabilistic sample of municipal primary care units in the city of *Rio de Janeiro*, Brazil (n=56). Managers (n=56), healthcare practitioners (n=541) and users (n=985) were interviewed.

Results

The mean score for the degree of implementation of the Breastfeeding-Friendly Primary Care Initiative in the study units was 5.45 (95%CI: 5.11 - 5.78), the maximum being 10.00. Existence of written guidelines and training obtained the worst scores. Breast massage and manual milk expression techniques; knowledge about

¹ Article based on the thesis of RVVF RITO, intitled "*Iniciativa Unidade Básica Amiga da Amamentação: avaliação da implantação em unidades da rede básica de saúde da cidade do Rio de Janeiro*". Fundação Oswaldo Cruz; 2009.

² Universidade Federal Fluminense, Faculdade de Nutrição. R. São Paulo, 30, Sala 407, *Campus Valonguinho*, Centro, 24020-150, Niterói, RJ, Brasil. *Correspondência para/Correspondence to:* RVVF RITO. *E-mail:* <rosane.rito@gmail.com>.

³ Universidade do Estado do Rio de Janeiro, Instituto de Nutrição. Rio de Janeiro, RJ, Brasil.

⁴ Universidade do Estado do Rio de Janeiro, Faculdade de Medicina. Rio de Janeiro, RJ, Brasil.

⁵ Fundação Oswaldo Cruz, Instituto Fernandes Figueira. Rio de Janeiro, RJ, Brasil.

⁶ Universidade de São Paulo, Faculdade de Saúde Pública. São Paulo, SP, Brasil.

contraception and the risks associated with the use of baby formulas, bottles, and pacifiers; and existence of support groups obtained intermediate scores. Provision of information and recommendations on breastfeeding rights and advantages; addressing users' worries, life experiences, and doubts; and encouraging cue feeding achieved the best scores.

Conclusion

The proposed tool assessed the general degree of implementation of the Breastfeeding-Friendly Primary Care Initiative and of each group of actions associated with the "Ten Steps", providing information for restructuring the strategies used in *Rio de Janeiro*. The degree of implementation of breastfeeding promotion, protection, and support set by the initiative in this city is intermediate, with its items having been implemented to different degrees.

Indexing terms: Breastfeeding. Breastfeeding-Friendly Primary Care Initiative. Primary healthcare.

RESUMO

Objetivo

Avaliar o nível de implantação da Iniciativa Unidade Básica Amiga da Amamentação em uma metrópole brasileira.

Métodos

Com base em protocolos validados, criou-se ferramenta de avaliação do nível de implantação composta por 55 parâmetros, gerando escore final que poderia variar de 0 a 10 pontos. Aplicou-se essa ferramenta em amostra probabilística das unidades básicas municipais de saúde da cidade do Rio de Janeiro (n=56). Foram entrevistados gestores (n=56), profissionais de saúde (n=541) e usuárias (n=985).

Resultados

A média do escore final das unidades básicas municipais de saúde referente ao nível de implantação da Iniciativa Unidade Básica Amiga da Amamentação foi de 5,45 (IC95%: 5,11 - 5,78). Observaram-se piores resultados em relação à norma escrita e ao treinamento. As técnicas de massagem e de ordenha das mamas, conhecimentos sobre contracepção, riscos do uso de fórmulas infantis, mamadeiras e chupetas e a implantação de grupos de apoio apresentaram resultados intermediários. As orientações sobre direitos, vantagens e recomendações relativas à amamentação, a escuta das preocupações, vivências e dúvidas da clientela e o encorajamento da amamentação sob livre demanda tiveram resultados mais satisfatórios.

Conclusão

A ferramenta desenvolvida permitiu a avaliação do nível geral de implantação da Iniciativa Unidade Básica Amiga da Amamentação e de cada conjunto de ações correspondentes aos "Dez Passos", fornecendo subsídios para reorientação das estratégias utilizadas na cidade. A cidade do Rio de Janeiro apresenta nível intermediário de implantação das ações de promoção, proteção e apoio à amamentação, preconizadas pela Iniciativa e pela heterogeneidade na implantação das atividades que a compõem.

Termos de indexação: Aleitamento materno. Iniciativa Unidade Básica Amiga da Amamentação. Atenção primária à saúde.

INTRODUCTION

Rated as one of the main actions fostering food safety¹, breastfeeding has been described as the best contributor to children's growth and development^{2,3}, and also benefits the mother's health^{4,5}. Despite its importance and the fact that 99.5% of Brazilian children begin breastfeeding on the first day of life, early weaning rates are still high in Brazil⁶.

Since the beginning of the 1980s, investments have been allocated to a nationwide policy designed to restore this practice on a wider scale by linking and mobilizing various segments of the Brazilian society⁷⁻⁹. In the state of *Rio de Janeiro*, the implementation of the Breastfeeding-Friendly Primary Care Initiative (BFPCI) began in 1999. This initiative, inspired by the Baby-Friendly Hospital Initiative (BFHI), was designed to

promote, protect and support breastfeeding at the primary healthcare level¹⁰.

The BFPCI is based on the “Ten Steps for Successful Breastfeeding in Primary Care”, which in turn is based on the assumption that prenatal care, childcare, and pediatrics provide a valuable opportunity for developing health-promoting activities and preventing and solving problems that may lead to early weaning. For this purpose, practitioner training is highly prioritized so that practitioners may implement standard and consistent actions based on scientific knowledge, actions that extend beyond the boundaries of biology and encompasses every aspect of lactating mothers^{11,12}.

The process of qualifying primary care units for the BFPCI was regulated in 2005¹³. This process requires that two external experts assess the BFPCI activities performed by a primary care unit. This assessment uses tested and validated protocols for analyzing documents from the prenatal and pediatric care services and includes interviews with unit managers, healthcare practitioners, pregnant women, and mothers of infants under one year of age. Ten sets of items are used for assessing compliance with the Ten Steps. The units are considered compliant with a step if at least 80% of its items are in effect. The title of Breastfeeding-Friendly Primary Healthcare Unit (BFPHU) is awarded for units that comply with all Ten Steps.

The Municipal Department of Health of *Rio de Janeiro* (SMS-RJ) started implementing the BFPCI in its primary healthcare units in 2000. Between 2003 and 2006, 69 courses were provided, qualifying more than 1,700 healthcare practitioners and community health agents. Seven years later, in 2007, the number of units awarded the BFPHU title became an indicator of the degree of BFPCI implementation in *Rio de Janeiro* because no tool was available to make a more thorough analysis. However, the number of units awarded this title (five) did not seem to fairly reflect the efforts made by the SMS-RJ to implement this initiative or the progress made by primary

healthcare units, evident during supervised activities. Seeking to overcome this gap and contribute to the advance of knowledge in this field, this study assessed the level of BFPCI implementation in the city of *Rio de Janeiro*.

METHODS

Instruments and criteria used for assessing the degree of BFPCI implementation

The tools used for assessing the degree of BFPCI implementation were the ten protocols used for awarding the BFPHU titles to the units, validated for this purpose¹². These protocols included document analysis, observation of prenatal and pediatric services, and interviews with primary care staff and users. In each primary care unit, a manager, ten healthcare practitioners, ten pregnant women, and ten mothers of infants under a year of age were interviewed.

These protocols were based on the “Ten Steps for Successful Breastfeeding in Primary Care”. They encompass structural aspects and written guidelines and routines (Step 1); professional training (Step 2); guidance on the duration of exclusive and non-exclusive breastfeeding, related advantages and legal rights (Step 3); boosting breastfeeding confidence (Step 4); breastfeeding lessons in the maternity hospital clinic (Step 5); suitable contraception while breastfeeding (Step 7); risks associated with using baby formulas and artificial nipples (Step 9); practical advice on latch on and position hand expression, and breastfeeding on demand (Steps 6 and 8); and organizing support groups and/or home visits (Step 10).

This study adapted the assessment criteria used for awarding the BFPHU title to identify the degree of BFPCI implementation. This means that instead of indicating no or full implementation, the results show the degree of implementation, which may vary from 0 to 10 points, the latter being full implementation.

The degree of BFPCI implementation reflects a unit's compliance with 55 items grouped into ten sets of activities that promote, protect, and support breastfeeding in primary care. Each set reflects each of the Ten BFPCI steps (set 1 reflects step 1 and so on). Each set is given a score from 0 to 1. The number of items in each set varied from three (Sets 4, 5, 6, and 8) to 11 (Set 3). Consequently, for these sets to be equivalent to the BFPCI steps, that is, for each set to contribute equally to the final score of a primary care unit, each set could have a maximum score of 1 point, and this point would be divided by the number of items in the set. For example, since set 1 consisted of 5 items, each item was worth 0.2 points. The final score, which varied from 0 to 10 points, was given by adding the scores of the ten sets.

Scores for items that required interviews were given by the number of completed interviews. Thus, all the study items were scored, even if, for example, not all practitioners of a unit were fully interviewed.

Chart 1 summarizes the 55 items grouped into ten sets and the associated maximum score. Different items in a set may rely on different sources for its data. For example, the items in set 9 rely on direct observation or interviews with managers, practitioners, pregnant women, and mothers.

Sampling

A probabilistic sample of the primary care network was implicitly stratified by regions, called planning areas, and the units within were classified according to the healthcare model they used and their size, resulting in three categories: traditional large (>162 visits), traditional small (<=162 visits) and Family Health Clinics/Community Health Agents Program (FHP/CHAP).

The unit records of 2007 were used for unit selection. The median monthly number of prenatal visits held at these units during the first

half of 2007 was used for sizing the traditional units (small and large). At that time there were 152 units: 79 traditional primary care units (38 large and 41 small) and 73 FHP or CHAP.

The final sample should consist of 56 primary care units, but an oversample of 3% was used to cover possible losses, totaling 58 units. This size was large enough to estimate the mean variable of interest (final score) with a maximum acceptable error of 5% at a confidence level of 95%, assuming that the center of the proposed scale (5) would be the expected (mean) value of the score and 1.2 would be the standard deviation of this mean, as these values were not known beforehand¹⁴.

The expansion factors for the sample corresponded to the inverse probability of including the units in the strata to which they were allocated.

Data collection and analysis

The units were assessed by two BFPCI experts trained by the State Department of Health and Civil Defense of *Rio de Janeiro* (SESDEC-RJ, Rio de Janeiro) who were, at that time, part of the staff of experts in charge of the accreditation processes. These experts were always from outside the planning area of the selected unit. Unit managers, healthcare practitioners, pregnant women, and mothers of infants under one year of age were interviewed. A specific questionnaire was used for each group (assessment protocols for BFPHU title awards, as detailed above).

Data were collected from November 2007 to May 2008 after meetings with representatives of the planning areas and selected units to inform them of the assessment process. The units were visited up to three times to complete the required number of interviews.

The general degree of BFPCI implementation and implementation of the sets related to the Ten Steps in the municipality of Rio de Janeiro were respectively estimated by the mean final score and

Chart 1. Assessment criteria for the degree of implementation of the Breastfeeding-Friendly Primary Care Initiative: items and their respective scores.

Set	Item	Continuation
		Score assigned to each item
1	1. Have written breastfeeding guidelines and routines.	0.2
	2. Provide a copy of these guidelines for assessment.	
	3. Number of BFPCI steps addressed by the guidelines.	
	4. Display the guidelines in at least two common areas frequented by pregnant women and mothers with babies.	
	5. Guidelines and routines are easy to understand by staff and users.	
2	1. The healthcare staff has been advised on the breastfeeding standards according to the primary care unit manager.	0.1
	2. Evidence of staff training in the mother-and-child sector.	
	3. The staff in the mother-and-child sector has a copy of the breastfeeding training program.	
	4. The training consists of at least 20 hours of lecture.	
	5. The training consists of at least 3 hours of supervised clinical practice.	
	6. Number of BFPCI steps encompassed by the training syllabus.	
	7. Proportion of trained practitioners working in the mother-and-child sector.	
	8. Proportion of practitioners who have worked in the mother-and-child sector for less than six months and still require training.	
	9. Proportion of randomly selected staff that has already been duly trained.	
	10. Proportion of interviewed practitioners who answered correctly at least 13 of the 15 specific questions on breastfeeding management and guidance.	
3	1. Existence of breastfeeding groups according to the unit heads.	0.091
	2. Report presented by the unit head with a minimum amount of educational activities at the prenatal and pediatric sectors.	
	3. Proportion of interviewed practitioners who knew about breastfeeding protection laws.	
	4. Proportion of interviewed practitioners who stated that babies should only be introduced to other fluids or foods after six months of age.	
	5. Proportion of interviewed practitioners who stated that babies must be breastfed for at least two years.	
	6. Proportion of interviewed pregnant women who knew two advantages of breastfeeding.	
	7. Proportion of interviewed pregnant women who had received counseling on exclusive or nonexclusive breastfeeding.	
	8. Proportion of interviewed mothers who had received counseling on exclusive breastfeeding.	
	9. Proportion of interviewed mothers whose child had not been prescribed any fluid or food before his sixth month of age except for medical reasons.	
	10. Proportion of interviewed mothers who had received counseling on nonexclusive breastfeeding for at least two years.	
	11. Proportion of interviewed mothers who were not given baby formula at the unit during the baby's first six months of life.	
4	1. Proportion of interviewed practitioners who knew mothers' common breastfeeding concerns or doubts and who had provided care that effectively increased mothers' confidence.	0.333
	2. Proportion of interviewed pregnant women who had received care and advice that increased their confidence.	
	3. Proportion of interviewed mothers who had received care and advice that increased their confidence.	
5	1. Proportion of interviewed practitioners who stated that breastfeeding must begin within the first hour of birth.	0.333
	2. Proportion of interviewed practitioners who mentioned at least two advantages of room sharing.	
	3. Proportion of interviewed pregnant women who had been informed of the importance of breastfeeding within the first hour after the birth and/or the importance of room sharing.	
6	1. Proportion of interviewed practitioners who explained how to maintain lactation and manually express milk, demonstrating the correct position by gripping the areola.	0.333
	2. Proportion of interviewed pregnant women who were taught how to maintain lactation; the correct position, latch on the areola and/or expressing milk manually.	
	3. Proportion of interviewed mothers who were taught how to maintain lactation; the correct position, latch on the areola and/or expressing milk manually.	

Chart 1. Assessment criteria for the degree of implementation of the Breastfeeding-Friendly Primary Care Initiative: items and their respective scores.

		Conclusion
Set	Item	Score assigned to each item
7	1. Proportion of interviewed practitioners who knew about the lactational amenorrhea method and the contraindications of exclusive breastfeeders taking the pill.	0.5
	2. Proportion of interviewed mothers who were informed about suitable contraceptive methods while breastfeeding.	
8	1. Proportion of interviewed practitioners who knew that breastfeeding on demand is a must.	0.333
	2. Proportion of interviewed pregnant women who had been encouraged to breastfeed on demand	
	3. Proportion of interviewed mothers who had been encouraged to breastfeed on demand.	
9	1. Users protected from the marketing of formulas and other baby foods, bottles, nipples, and pacifiers, according to the unit heads.	0.111
	2. Proportion of interviewed practitioners who reported that the unit was not given samples of infant formulas.	
	3. Proportion of interviewed practitioners who knew that bottles pose a risk to breastfeeding.	
	4. Proportion of interviewed practitioners who knew that pacifiers pose a risk to breastfeeding.	
	5. Proportion of interviewed pregnant women who had been informed about the risk of using bottles or pacifiers.	
	6. Proportion of interviewed mothers who had been informed about the risk of using bottles or pacifiers.	
	7. Experts found no advertising or distribution of baby formulas, bottles, nipples, or pacifiers, or any sample of these products in the unit.	
	8. Experts found no representatives or salesmen of the baby food or nipple industries at the unit.	
	9. Experts found that the products found in the unit storage designed for pregnant women or babies were medically appropriate, within their expiry dates, and compliant with the Brazilian Act on the Sale of Foods, Nipples, Pacifiers, and Bottles for Breastfeeding Mothers and Young Babies.	
10	1. Unit head confirmed that pregnant women and mothers received breastfeeding advice in groups or during home visits.	0.166
	2. Proportion of interviewed pregnant women who participated in group activities or were visited at home, where they exchanged experiences and received breastfeeding advice.	
	3. Proportion of interviewed mothers who participated in group activities organized by the unit or were visited at home, where they exchanged experiences and received breastfeeding advice.	
	4. Proportion of interviewed pregnant women with at least one family member who had been invited to participate or was present at some activity (group, visit, home visit or other) organized by the unit that included breastfeeding advice.	
	5. Proportion of interviewed mothers with at least one family member who had been invited to participate or was present at some activity (group, visit, home visit or other) organized by the unit that included breastfeeding advice.	
	6. Proportion of interviewed mothers who had been told to revisit the unit whenever they had a breastfeeding problem to receive the necessary support without the need of appointments.	

Note: PCU: Primary Care Unit; BFPCI: Breastfeeding-Friendly Primary Care Initiative.

the mean score of each of the ten sets of all the study units. The means and their respective 95% confidence intervals (95%CI) were estimated with the sampling design in mind. The set scores are presented by a scatter box plot. The final scores by planning area and unit classification were compared by examining the 95%CI of the estimates. The differences were considered

significant when the CI of the compared groups did not overlap. To reduce the number of categories for each variable, the planning areas were regrouped into five regions by geographical proximity, and the units were regrouped by care model: traditional and family health strategy.

The data were entered in the EPI-DATA 3.4 software and the item scores, set scores, and

final primary care unit scores were calculated by the software Statistical Package for the Social Sciences (SPSS), version 13.

This study is in compliance with Resolution nº 196/96 issued by the National Health Council (CNS), which establishes the guidelines and regulations for research on human beings. The study was approved by the Research Ethics Committee of the Municipal Health Department and Civil Defense Force of Rio de Janeiro, under protocol number 158a/2007.

RESULTS

A total of 56 units were assessed, of which 16 were large traditional units, 12 were small traditional units, and 28 were FHP/CHAP units. Two initially sampled units were not studied: one of them (CHAP) was deactivated and the other (FHP) could not be visited because of safety reasons, despite several attempts. A total of 56 unit managers, 541 practitioners, 485 pregnant women, and 500 mothers were interviewed, 100.0%, 96.6%, 86.6%, and 89.3% of the planned interviews, respectively.

The mean final score of the units for degree of BFPCI implementation in the municipality of *Rio de Janeiro* was 5.447 (95%CI:

5.111-5.784) (Table 1). Sets 1 and 2 had the worst scores: 0.258 and 0.403, respectively; they regarded the provision and display of breastfeeding guidelines and routines, and the training of human resources.

The following five sets had average scores, varying from 0.479 to 0.632: set 9 (risks associated with using baby formulas, feeding bottles and pacifiers); set 10 (breastfeeding support groups); set 6 (breastfeeding management techniques); set 7 (appropriate contraceptive methods while breastfeeding); and set 8 (encourages breastfeeding on demand).

Table 1. Mean scores of each set of Breastfeeding-Friendly Primary Care Initiative items and mean final scores of the study primary care units. *Rio de Janeiro* (RJ), Brazil, 2008.

Variables	Mean	95% confidence interval
Sets		
1	0.258	0.174 - 0.342
2	0.403	0.338 - 0.468
3	0.706	0.675 - 0.737
4	0.722	0.678 - 0.765
5	0.763	0.733 - 0.792
6	0.497	0.453 - 0.541
7	0.500	0.457 - 0.542
8	0.632	0.599 - 0.665
9	0.479	0.458 - 0.500
10	0.489	0.446 - 0.532
Final Score	5.447	5.111 - 5.784

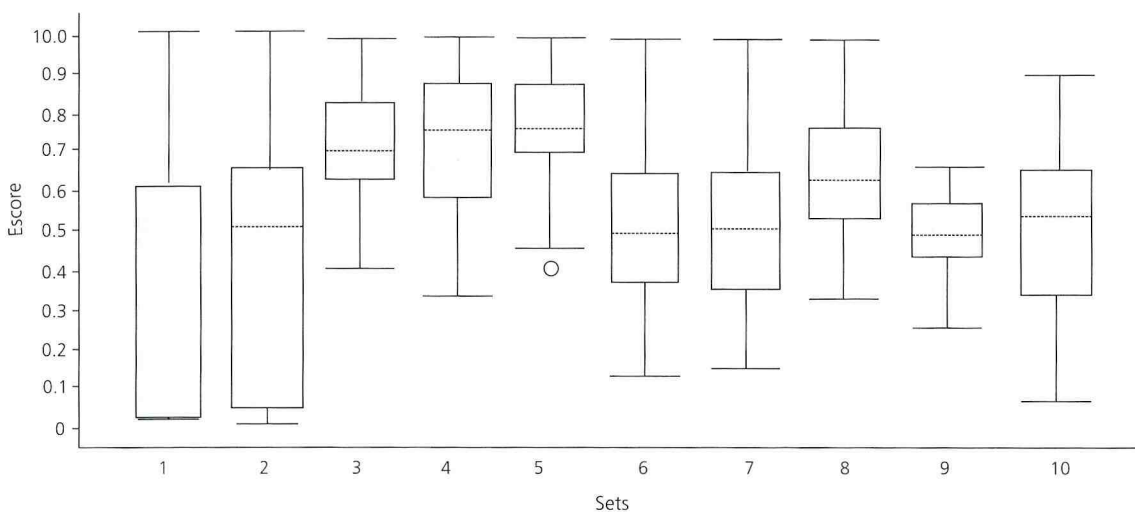


Figure 1. Scatter box plot showing the scores of each set of items used for assessing the Breastfeeding-Friendly Primary Care Initiative. *Rio de Janeiro* (RJ), Brazil, 2008.

Table 2. Mean final score of the Breastfeeding-Friendly Primary Care Initiative implementation by region and by primary care unit care model. *Rio de Janeiro (RJ), Brazil, 2008.*

Variables	N° primary care units	Mean	95% confidence interval
<i>Regions</i>			
1	4	4.294	4.160 - 4.427
2	7	5.471	4.897 - 6.045
3	16	5.174	4.526 - 5.821
4	4	5.954	4.601 - 7.308
5	25	5.762	5.203 - 6.322
<i>Care Model</i>			
PCU	28	5.429	4.998 - 5.860
FHS	28	5.466	4.947 - 5.985

Note: PCU: Primary Care Units with traditional care models; FHS: Family Health Strategy (healthcare clinics run by the FHS/Community Health Agents Program).

The following sets had the best scores, ranging from 0.706 to 0.763: set 3 (informing mothers of their breastfeeding rights, the advantages of breastfeeding, and related recommendations); set 4 (supporting and boosting mothers' confidence); and set 5, (practitioners' and pregnant women's knowledge of procedures that encourage breastfeeding during the neonatal period).

Figure 1 shows the degrees of implementation of the ten sets by the study units. The degree of implementation of sets 3, 5, 8, and 9 by all the units was similar, while that of sets 4, 6, 7, and 10 varied to some extent, and that of sets 1 and 2 varied mostly.

Comparison of the five regions showed that region 1 had the worst degree of BFPCI implementation, but its final score did not differ significantly from the mean score of the other four study regions. When the units were grouped by care model, the mean final scores were also similar (Table 2).

DISCUSSION

The degree of BFPCI implementation in the municipality of *Rio de Janeiro* was intermediate, and region 1 obtained the worst score. These findings reflect the fact that region 1 had invested

the least in professional training for promoting breastfeeding and for BFPCI implementation, as shown by SMS-RJ's Annual Report on Actions Promoting, Protecting and Supporting Breastfeeding of 2008, kindly provided by their Child Healthcare Program Coordination.

Less than one third of the study units had written breastfeeding guidelines, despite this having been determined by the Ministry of Health. This procedure is essential for building an institutional legacy and for actual breastfeeding¹⁵. These findings confirm those reported by Toma & Monteiro¹⁶ when they used Baby-Friendly Hospital Initiative criteria to assess breastfeeding promotion in 45 maternity hospital clinics in the municipality of *São Paulo*. These criteria also require the availability of printed copies of the breastfeeding guidelines but the authors noted difficulties in compliance with this step, as these printed copies were only available to the healthcare staff in a minority of public hospitals and in no private hospitals.

Despite the efforts of the SMS-RJ managers to provide training courses, set 2, which includes staff training, obtained the second lowest mean score. This poor performance may be explained by the number of practitioners who still required training, since *Rio de Janeiro* - Brazil's second largest metropolis - has numerous primary care units employing thousands of practitioners.

In other words, many courses are necessary to ensure high training penetration rate.

The small number of trained professionals corroborates the findings of the first assessment of compliance with the Ten Steps for Successful Breastfeeding at Baby-friendly Hospitals done in Brazil¹⁷. On that occasion, the implementation of training courses was also small, confirming the need to assign high priority to training to improve advice quality and effective support for pregnant women and new mothers. However, the existence of a practitioner training policy that blends the precepts of critical and reflective education, ongoing healthcare education, and problematization¹⁷, resulting in appropriate support for mothers during the prenatal, natal, and postnatal periods, is an important requirement for increasing breastfeeding prevalence¹¹. Caldeira *et al.*¹⁸ noted that the family health team training proposed by the BFPCI proved to be an effective, inexpensive strategy for increasing practitioner awareness, helping them to provide more homogeneous information and better support to mothers with breastfeeding difficulties.

The sets with intermediate degrees of implementation, which consequently warrant more attention during the training sessions, were: showing women how to breastfeed and maintain milk production; providing guidance on appropriate contraception while breastfeeding and on the risks associated with the use of artificial nipples; and strengthening the social support network for breastfeeding. Recent studies reiterate the need for investments in this area, especially practitioner training. When studying infant growth, Jaldin *et al.*² pointed out the importance of using the right breastfeeding techniques, emptying the breast completely at each feed, and explaining that the milk produced at the end of each feed is higher in fats and energy, resulting in satiety and greater weight gain; they also stress that constant support for mothers during childcare is crucial for boosting mothers' self-esteem, making them believe in

their ability to breastfeed. Meanwhile, Roig *et al.*¹⁹ warn of the need to disseminate knowledge about the use of bottles and pacifiers and their association with weaning in the first six months of life.

In general, knowledge about the advantages, rights, and recommendations on exclusive and non-exclusive breastfeeding (set 3) was well absorbed by the study practitioners and mothers. Similarly, knowledge about breastfeeding during the first hour of life and about the importance of room sharing and breastfeeding on demand (set 5) was satisfactory. Furthermore, set 4 (which addresses the reception given to pregnant and breastfeeding women and the boosting of their confidence by listening to their breastfeeding-related concerns, experiences, and doubts) was much better implemented than nearly all other sets (except for set 5).

In general lines, our findings converge with those of Cruz *et al.*²⁰ with respect to the guidelines best incorporated by primary care practitioners.

Cruz *et al.*²⁰ investigated what breastfeeding-related advice women with children aged two years or less had received in primary care units and found that more than 70% had been advised to start breastfeeding within the first hour of life. Additionally, they were informed about the advantages of exclusive breastfeeding for six months and about the importance of breastfeeding on demand and suction for milk production. Smaller proportions (between 59% and 68%) were taught how to manually express milk and position themselves and the child; advised to breastfeed until the child was two years old or older; and informed of the harm caused by pacifiers and bottles and of breastfeeding difficulties.

However, our findings differ from those of Cruz *et al.*²⁰ regarding the difference between primary care units that follow a traditional care model and family health units: while our study found no statistically significant differences between the units grouped by care model, Cruz *et al.*²⁰ found that women seen at family health

units were more likely to have received all the breastfeeding advice than those seen at units that follow the traditional model. The different degrees of practitioner training found by the two studies may explain this discrepancy. Cruz *et al.*²⁰ hypothesized that this difference by care model stemmed from the more recent training given to practitioners from family health units. However, at the time of the present study, training activities associated with BFPCI implementation had been given to practitioners in all healthcare units, regardless of care model.

In terms of internal validity, the present study assessed the units with the same tools as those used by experts to award BFPHU titles. During the final phase of the fieldwork, one of the units was excluded because of urban violence and it could not be replaced. A second unit was closed but we decided not to replace it because the sample size could accommodate these two losses.

As mentioned earlier, number of items per set varied, as did the scores attributed to each item. However, all sets were given equal weight in the final score to reflect the BFPHU title-awarding process, where all ten steps have the same weight.

In terms of external validity, the assessment method used by the present study may be used for assessing actions that promote, protect, and support breastfeeding in primary care units of other municipalities that strive to follow the study sets.

In summary, the primary care units of *Rio de Janeiro* were able to implement many practices that promote breastfeeding, encouraging practitioners to promote them too. On the other hand, managers need to review the resources available at the central, regional, and local levels, such as the creation and dissemination of breastfeeding guidelines and routines, and professional training.

The present study demonstrated the importance of developing a tool for assessing the implementation of policies that promote

breastfeeding, such as the BFPCI; a tool that does not rely only on the number of units awarded the BFPCI title and that allows the identification of aspects critical for improving this implementation. In addition to assessing healthcare as a whole, at the municipal or regional levels, the proposed tool allows each primary care unit manager to examine the specific reality of his unit and perform peer reviews of the implementation process at the local level.

The consolidation of a breastfeeding culture depends on numerous factors. To improve the knowledge about this process, this study proposes an innovative tool capable of better identifying the dynamics of breastfeeding promotion done by the healthcare sector.

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CONTRIBUTORS

RVVF RITO and IRR CASTRO contributed to the concept and design of the study, data collection, analysis, and interpretation, and manuscript writing and final review. AJB TRAJANO and MASM GOMES contributed to project conception and manuscript. RTI BERNAL contributed to sampling design, data analysis, and to the manuscript.

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Processed foods aimed at children and adolescents: Sodium content, adequacy according to the dietary reference intakes and label compliance¹

Alimentos processados voltados para crianças e adolescentes: concentração de sódio, adequação em relação aos níveis de ingestão dietética de referência e conformidade da rotulagem

Vera Favila RIBEIRO²

Marisilda de Almeida RIBEIRO³

Margarida Angélica da Silva VASCONCELOS⁴

Samara Alvachian Cardoso ANDRADE⁵

Tânia Lúcia Montenegro STAMFORD⁴

ABSTRACT

Objective

This study determined the sodium content of processed foods aimed at children and adolescents and the adequacy of its content in relation to the dietary reference intakes, and verified label compliance.

Methods

The sodium content of 17 food samples (instant noodles, breaded items, hamburger patties, hot dogs and bologna sausages) was determined by flame photometry and chloride titration, and the results were compared with nutritional data. The labels were checked for compliance with the pertinent laws.

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² Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Núcleo de Nutrição. Av. Prof. Moraes Rego, 1235, Cidade Universitária, 50670-901, Recife, PE, Brasil. *Correspondência para/Correspondence to:* VF RIBEIRO. *E-mail:* <vfavila@hotmail.com>.

³ Universidade Federal de Pernambuco, Departamento de Nutrição. Vitoria de Santo Antão, PE, Brasil.

⁴ Universidade Federal de Pernambuco, Centro de Ciências da Saúde, Departamento de Nutrição. Recife, PE, Brasil.

⁵ Universidade Federal de Pernambuco, Centro de Tecnologia, Departamento de Engenharia Química. Recife, PE, Brasil.

Results

According to flame photometry and chloride titration, 13 and 5 products, respectively, had sodium contents that exceeded those reported on the nutrition facts label by more than 20%. All samples had more than 480mg of sodium per serving. The tolerable upper intake level for sodium for children aged 4-8 years was exceeded in 8 instant noodles and 3 breaded items according to flame photometry, and in 9 items according to chloride titration. Regarding the legislation, 5 products used a daily reference intake other than that provided by the legislation to report their percent sodium content per serving. Moreover, the serving sizes of 3 instant noodles, the terminology used in 1 instant noodles and the protein content of 1 breaded item were also not compliant with the legislation.

Conclusion

The sodium contents in the study samples were high and there was no regard for the legislation.

Indexing terms: Food labeling. Processed foods. Sodium chloride.

RESUMO

Objetivo

Determinar o teor de sódio em alimentos processados voltados para crianças e adolescentes, averiguar a adequação do teor encontrado em relação aos níveis de ingestão dietética de referência e verificar a conformidade das informações veiculadas na rotulagem.

Métodos

Análise experimental do teor de sódio em 17 amostras (massa instantânea, empanado, hambúrguer, salsicha e mortadela) pela fotometria de chama e determinação de cloretos por volumetria. Os resultados foram comparados aos dados das informações nutricionais. A conformidade da rotulagem foi verificada a partir das legislações pertinentes.

Resultados

Houve variação maior que 20% entre o teor de sódio analisado e o declarado em 13 produtos pela fotometria de chama, e em 5, pela volumetria. Todas as amostras apresentaram mais de 480mg de sódio/porção e o limite de ingestão adequada para crianças de 4 a 8 anos foi ultrapassado, pela fotometria, nas 8 massas instantâneas e em 3 empanados. Pela volumetria, a oferta de sódio ultrapassa os níveis de ingestão recomendados para crianças entre 4 e 8 anos em 9 amostras analisadas. Em relação à legislação: 5 amostras apresentaram desacordos nos percentuais de referência estabelecidos; a regulamentação das porções dos alimentos foi descumprida por 3 massas instantâneas; 1 produto dessa categoria utilizou-se de denominação incorreta; e 1 empanado apresenta teor proteico menor que o estabelecido.

Conclusão

Verificou-se elevado teor de sódio nos alimentos e desrespeito às legislações vigentes.

Termos de indexação: Rotulagem de alimentos. Alimentos processados. Cloreto de sódio.

INTRODUCTION

In the last decades, the consumption of processed foods such as instant noodles, hot dogs, bologna sausage, hamburger patties and breaded items has increased, mainly because of their fast preparation and affordability. Their taste, obtained with food ingredients and additives, is also appealing to most consumers who use them in place of or along with the main meals, especially meals for children and adolescents.

In order to meet the demand for these products, the food industry offers the convenience desired by adults and attractive packaging for the youth. However, the addition of sodium to processed foods should be monitored, justifying the required nutrition facts label.

The increasing consumption of processed foods along with the addition of salt during cooking and at the table are the main causes of the excessive sodium intake of the Brazilian

population^{1,2}. Consequently, the Ministry of Health presented a Plan for Fighting Chronic Non-communicable Diseases (CNCD), which includes actions to promote healthy diets and includes a goal to reduce the mean salt consumption of 12g to 5g by 2022³.

In order to reach these goals, Ministry of Health and many food industry associations began signing agreements in 2011 to reduce the sodium content of many foods, such as instant noodles, breads, cakes, corn chips, potato chips, mayonnaise, sweet and savory cookies, breakfast cereals and seasonings⁴.

These government actions are very important, but since the nutrient content reported on the label can stem from chemical analyses or calculations based on product formulation⁵, and since the Brazilian legislation allows the contents reported on the label and those determined analytically to differ by as much as 20%⁶, it is important to check the accuracy of the reported data.

In addition to the nutrition facts label and other compulsory information, each product has particularities that must comply with various resolutions and normative instructions, and the packaging is the consumer's information source. Therefore, it is essential to verify if the legislation is being followed.

The objectives of this study were to determine the sodium content of processed foods whose packaging aims at children and adolescents, verify if their sodium contents are in agreement with the daily reference intakes and verify if the information on the label is in conformity with the legislation.

METHODS

The study food items were chosen based on *Agência Nacional de Vigilância Sanitária's* (Anvisa, National Sanitary Surveillance Agency) Technical Communication n° 42/2010⁷, which contains the nutritional profile of some processed foods with high sodium, saturated fat and sugar

contents that are usually consumed by the Brazilian population. The inclusion criteria were the possibility of using the food to replace or complement a meal, and the presence of elements on the packaging that appealed to children and adolescents.

Seventeen food items were analyzed, namely eight instant noodles from three different brands, one hamburger patty, one bologna sausage, two hot dogs from two different brands and five breaded items from three different brands.

Once the samples were acquired, the nutrition facts labels were recorded in a form created for this purpose and compared with the resolutions provided by the executive board of directors of Anvisa^{6,8-10} and with the normative instructions provided by the *Ministério da Agricultura e Pecuária* (Ministry of Agriculture, Livestock and Supply)¹¹⁻¹³.

The raw samples were ground and homogenized by a food processor for approximately 90 seconds. The seasonings of the noodle samples were homogenized together with the noodles. An aliquot of 5g of each sample was placed in a porcelain capsule previously desiccated and weighed by an analytical scale of the brand Sartorius BL2105. The samples were burned by a Bunsen burner until no more smoke was emitted.

The ashes were obtained as recommended by the Association of Official Analytical Chemists¹⁴ (AOAC). Sodium content was quantified by flame photometry as recommended by the AOAC¹⁵ and the chloride in sodium chloride was quantified by the method 028/IV of Institute Adolfo Lutz¹⁶, where the sodium content is estimated to be 40% of the amount of chloride found.

These quantitative techniques were chosen because of the high sodium contents normally found in processed foods and because chloride titration and sodium quantification by flame photometry are official methods used by the Anvisa.

In order to verify the adequacy of the sodium content, the sodium content per serving reported on the label and that determined

analytically were compared with the Adequate Intake (AI) values for children and adolescents aged 4-8 and 9-18 years and with the tolerable Upper Intake Levels (UL) for children and adolescents aged 4-8, 9-13 and 14-18 years¹⁷.

The experimental results were submitted to Analysis of Variance (Anova) and compared by the Duncan test, using a probability level of 5%. The statistical treatment was done by the software Statistica for Windows 7.0".

RESULTS

Table 1 shows the food characteristics and marketing strategies present on the labels and the reported sodium contents of the 17 study foods.

The label contents of 5 of the 17 study products did not comply with the legislation, violating norms RDC n° 360/3⁶, RDC n° 359/3¹⁰, RDC n° 263/5⁸ and IN n° 6/1¹¹.

The labels of all study products were in compliance with the legislation regarding the listing of nutrients and respective percentages of their daily requirements. However, 5 did not use the established daily reference intakes. The nutrition facts label of the study hamburger patty reported a sodium content of 629mg of sodium per serving, which, according to the said label, corresponds to 13% of the daily reference intake. However, if the considered daily reference intake is 2,400mg of sodium⁶, the reported amount corresponds to 26% of the daily reference intake.

Three instant noodles also used daily reference intakes different from those provided by the legislation. The percentages of proteins and vitamins B₁, B₂, B₃ and B₆ in the instant noodles D and E refer to the requirements of children aged 7-10 years, while the percentages of the same vitamins in the instant noodles H refer to the requirements of children aged 4 to 6 years. The other nutrients use the daily intake references provided by the resolution⁶.

Table 1. Characteristics, marketing strategies present on the packaging and sodium contents of ready-to-eat and fast foods aimed at children and adolescents. Recife (PE), Brazil, 2011.

Product	Characteristics		Marketing strategies				Sodium content (mg/100g)		
	Flavor	Shape	TV character	Colorful letters	Games	Other*	Label	Flame photometry	Titration
InsNood A	Beef	Standard	Yes	Yes	No	Yes	2351.75 ^a	2556.71 ^a	1896.18 ^a
InsNood B	Chicken	Standard	Yes	Yes	No	Yes	2310.59 ^a	2184.99 ^a	2166.94 ^a
InsNood C	Tomato	Standard	Yes	Yes	No	Yes	2014.12 ^b	2771.96 ^a	1722.81 ^b
InsNood D	Beef	Standard	Yes	Yes	No	Yes	2387.50 ^a	2532.96 ^a	1786.95 ^b
InsNood E	Chicken	Standard	Yes	Yes	No	Yes	2384.40 ^a	2801.98 ^a	2007.05 ^a
InsNood F	Mild beef	Standard	No	Yes	No	Yes	1642.30 ^b	2301.78 ^a	1702.29 ^b
InsNood G	Mild chicken	Standard	No	Yes	No	Yes	1990.60 ^b	2776.31 ^a	1781.91 ^b
InsNood H	Beef	Standard	Yes	Yes	No	Yes	2133.00 ^{ab}	2574.95 ^a	1786.63 ^b
BriItem A	Chicken	Standard	Yes	No	Yes	Yes	400.00 ^c	980.21 ^a	778.03 ^b
BriItem B	Chicken	Varied	Yes	Yes	Yes	Yes	400.00 ^c	927.37 ^a	633.56 ^b
BriItem C	Chicken w/carrot	Standard	Yes	No	Yes	Yes	400.00 ^b	899.57 ^a	810.02 ^a
BriItem D	Fish	Seafood	No	Yes	No	Yes	453.00 ^c	847.73 ^a	665.29 ^b
BriItem E	Chicken	Stars	No	No	No	Yes	708.00 ^b	1448.31 ^a	1264.60 ^a
HPatty A	Chicken	Standard	Yes	No	Yes	Yes	786.00 ^b	1094.99 ^a	861.41 ^b
Hot dog A	Chicken	Standard	Yes	No	No	Yes	1180.00 ^b	1685.28 ^a	1261.44 ^b
Hot dog B	Chicken	Miniature	No	Yes	No	Yes	936.00 ^b	1553.69 ^a	881.27 ^b
BolSau A	Chicken	Standard	Yes	No	No	Yes	1180.00 ^b	2009.75 ^a	1246.81 ^b

Note: InsNood: Instant Noodles; BriItem: Breaded Items; HPatty: Hamburger Patty; BolSau Bologna Sausage.

Measurements in the same line followed by same letters are not significantly different at the 5% significance level according to the Duncan test.

*Made especially for children and adolescents; mild flavor; consumption should accompany decorated dishes to encourage children to eat; information about sites with games; drawings of characters, animals or mascots on the packaging; information encouraging children to practice physical activities; easy and fast to prepare; contains vitamins and other nutrients.

Companies may include other nutrients on the nutrition facts label if they exceed 5% of the Daily Reference Intake (DRI)⁶ per the serving size indicated on the label. The breaded item D incorrectly reports calcium. Its content of 23.0mg per serving corresponds to only 2% of the DRI, not 5%, which would be equivalent to 57.5mg.

The serving sizes reported on the labels of three instant noodles did not comply with the serving size of this type of food provided by the legislation, which is of 80g. The packaging of the instant noodle H contained 80g of product, but the reported serving size was 33g, and the reported nutrient contents were for this serving size. On the other hand, the packaging of the instant noodles D and E did not contain 80g but 64g, that is, less than the amount required by the legislation¹⁰.

The technical norms for the denomination of grain and starchy products, flours and brans⁸ were also violated. The instant noodle H consisted of wheat flour and cassava starch and called itself instant noodle. However, according to the legislation, noodles are made exclusively from wheat flour and/or durum wheat derivatives. When another source of starch is added, the product is classified as a dough mix.

The identity and quality standard for breaded products requires a minimum protein content of 10%¹¹. The breaded item D had 22.0g of protein, representing a protein content of 9%. Therefore, it was not in compliance with the law, since it should contain at least 24.4g of protein.

Figure 1 shows that the difference between the reported and quantified sodium

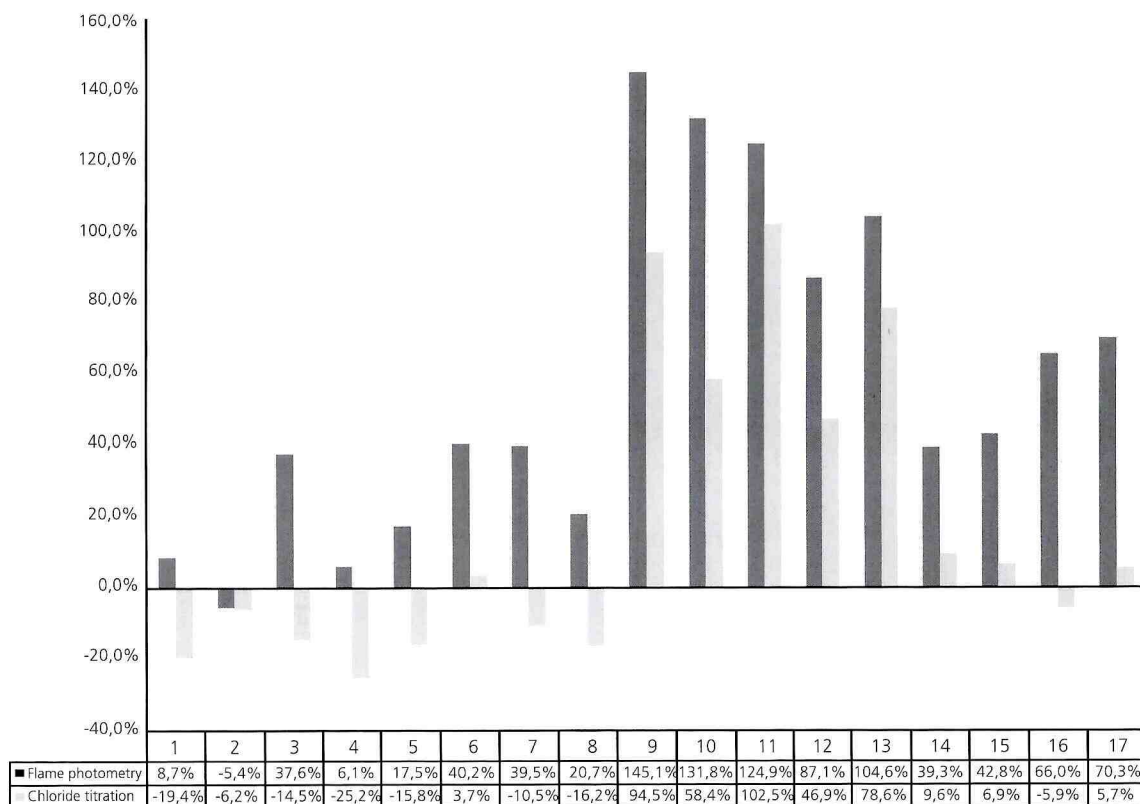


Figure 1. Percentage variation between the sodium contents reported on the labels and those found analytically by flame photometry and titration. Recife (PE), Brazil, 2011.

Note: 1: Instant noodles A; 2: Instant noodles B; 3: Instant noodles C; 4: Instant noodles D; 5: Instant noodles E; 6: Instant noodles F; 7: Instant noodles G; 8: Instant noodles H; 9: Breaded item A; 10: Breaded item B; 11: Breaded item C; 12: Breaded item D; 13: Breaded item E; 14: Hamburger patty A; 15: Hot dog A; 16: Hot dog B; 17: Bol sausage A.

contents exceeded 20% in 13 products analyzed by flame photometry and 5 analyzed by chloride titration. Therefore, these products are not in compliance with the norm RDC 360/03⁶.

Based on flame photometry, all 17 samples are high in sodium since all of them have more than 480mg of sodium per serving¹⁸. Eight instant noodle samples and three breaded items analyzed by flame photometry exceeded the UL for sodium for children aged 4-8 years. Additionally, all 17 study products had sodium contents in excess of 50% of the UL for all age groups. Finally, the UL for sodium for children aged 4-8 years was exceeded by six instant noodle samples (Table 2).

Table 3 compares the sodium contents per serving determined by chloride titration with the sodium DRI for children and adolescents. According to this method, only hot dog B was not classified as a high-sodium food¹⁸. The sodium contents of 9 study products exceeded the UL for children aged 4-8 years and all 8 instant noodles provided more than 50% of the UL for all age groups.

DISCUSSION

Since the nutritional data reported by the food industry can be determined by chemical analyses and calculations based on the formulation of the product⁵, nutrient contents may differ. These different methods may partially explain the difference between the contents reported on the label and those found analytically by the present study, which, according to the legislation, should not exceed 20%⁶.

Thirteen samples analyzed by flame photometry and 5 by chloride titration exceeded the tolerated difference (Figure 1). Other studies found differences in sodium contents in excess of 20% in 5 (50%) pastas¹⁹ and 64 (57%) snack and potato chips²⁰, corroborating the results of the present study. These excessive differences show that it is essential to effectively survey all nutrition information reported on the labels to check their accuracy, allowing well-informed

Table 2. Comparison between the sodium content according to flame photometry and the daily reference intakes^a for children and adolescents. Recife (PE), Brazil, 2011.

Product	Na (mg)/serving ^b	% AI adequacy/serving		% UL adequacy/serving		
		4-8 years ^c	9-18 years ^d	4-8 years ^e	9-13 years ^f	14-18 years ^g
InsNood A	2045.37	170.4	136.4	107.6	92.9	88.9
InsNood B	1747.99	145.7	116.5	92.0	79.4	76.0
InsNood C	2217.57	184.8	147.8	116.7	100.8	96.4
InsNood D	2026.37	168.9	135.1	196.7	92.1	88.1
InsNood E	2241.58	186.8	149.4	117.9	101.9	97.5
InsNood F	1841.42	153.4	122.8	96.9	83.7	80.1
InsNood G	2221.05	185.1	148.1	116.9	100.9	96.6
InsNood H	2059.96	171.7	137.3	108.4	93.6	89.6
Brltem A	1274.27	106.2	84.9	67.1	57.9	55.4
Brltem B	1205.58	100.5	80.4	63.4	54.8	52.4
Brltem C	1169.44	97.4	78.0	61.5	53.1	50.8
Brltem D	1102.05	91.8	73.5	58.0	50.1	47.9
Brltem E	1882.80	156.9	125.5	99.1	85.6	81.9
HPatty A	875.99	73.0	58.4	46.1	39.8	38.1
Hot dog A	842.64	70.2	56.2	44.3	38.3	36.6
Hot dog B	776.85	64.7	51.8	40.9	35.3	33.8
BolSau A	803.90	67.0	53.6	42.3	36.5	34.9

Note: InsNood: Instant Noodles; Brltem: Breaded Items; HPatty: Hamburger Patty; BolSau: Bologna Sausage; AI: Appropriate Intake; UL: Upper Intake Level.

^aFood and Nutrition Board/National Research Council (2004). ^bServing sizes according to the norm RDC n° 359/2003: Hamburger patty=80g; Breaded item=130g; Instant noodles=80g; Bologna sausage=40g; Hot dog=50g. ^cAI: 1.2g/day. ^dAI: 1.5g/day. ^eUL: 1.9g/day. ^fUL: 2.2g/day. ^gUL: 2.3g/day.

Table 3. Comparison between the sodium content according to chloride titration and the daily reference intakes^a for children and adolescents. *Recife* (PE), Brazil, 2011.

Product	Na (mg)/serving ^b	% AI adequacy/serving		% UL adequacy/serving		
		4-8 years ^c	9-18 years ^d	4-8 years ^e	9-13 years ^f	14-18 years ^g
InsNood A	1516,94	126,4	101,1	79,8	68,9	65,9
InsNood B	1733,55	144,5	115,6	91,2	78,8	75,4
InsNood C	1378,25	114,8	91,9	72,5	62,6	59,9
InsNood D	1429,56	119,1	95,3	75,2	64,9	62,1
InsNood E	1605,64	133,8	107,0	84,5	72,9	69,8
InsNood F	1361,83	113,5	92,1	71,7	61,9	59,2
InsNood G	1425,53	118,8	95,0	75,0	64,8	61,9
InsNood H	1429,30	119,1	95,3	75,2	64,9	62,1
Brltem A	1011,44	84,3	67,4	53,2	45,9	43,9
Brltem B	823,63	68,6	54,9	43,3	37,4	35,8
Brltem C	1053,03	87,8	70,2	55,4	47,9	45,8
Brltem D	864,88	72,1	57,6	45,5	39,3	37,6
Brltem E	1643,98	136,9	109,6	86,5	74,7	71,5
HPatty A	689,13	57,4	45,9	36,3	31,3	29,9
Hot dog A	630,72	52,6	42,0	33,2	28,7	27,4
Hot dog B	440,64	36,7	29,4	23,2	20,0	19,1
BolSau A	498,72	41,6	33,2	26,2	22,7	21,7

Note: InsNood: Instant Noodles; Brltem: Breaded Items; HPatty: Hamburger Patty; BolSau: Bologna Sausage; AI: Appropriate Intake; UL: Upper Intake Limit.

^aFood and Nutrition Board/National Research Council (2004). ^bServing sizes according to the norm RDC n° 359/2003¹⁰: Hamburger patty=80g; Breaded item=130g; Instant noodles=80g; Bologna sausage=40g; Hot dog=50g. ^cAI: 1.2g/day. ^dAI: 1.5g/day. ^eUL: 1.9g/day. ^fUL: 2.2g/day. ^gUL: 2.3g/day.

Table 4. Variation of the sodium content of same-category foods. *Recife* (PE), Brazil 2011.

Product	Mean ^a Na (mg)/serving ^b	Difference between the maximum and minimum contents found (n° of times)	Country
Instant noodles	2721	2,25	Brazil ^c
Breaded chicken	759	1,86	
Poultry hamburger patty	525	2,6	
Hot dog	551	2,0	
Instant noodles	413	14,5	Austrália ^d
Hamburger patty	384	19,0	
Hot dog	412	9,4	
Breaded chicken	407	2,1	New Zealand ^e
Hamburger patty	368	1,9	
Hot dog	426	1,9	
Instant noodles	2178	1,3	Brazil ^f
Breaded chicken and fish	1326	1,7	
Chicken hot dog	809	1,5	
Instant noodles	1528,3	1,5	Brazil ^g
Beef hamburger patty	560,8	8,4	
Chicken bologna sausage	492,8	1,6	

Note: ^aMean contents found by flame photometry; ^bInstant noodles: 85g; Breaded items: 130g; Hamburger patty: 80g; Hot dog: 50g; Bologna sausage:40g; ^cAnvisa⁷; ^dWebster et al.²² ^eThomson²¹. ^fData found by the present study. ^gAnvisa⁸.

consumers to correctly choose the most appropriate food item among all those available.

Comparison of the sodium contents reported on the labels with those determined analytically shows that the differences between both are significant ($p < 0.05$) in 12 samples analyzed by flame photometry and 6 samples analyzed by chloride titration. Many factors may influence the results of the chemical analyses, such product formulation and analytical method. The determination of chlorides by titration assesses salinity¹⁶ while flame photometry determines the content of sodium ions directly¹⁵. This may explain the differences between the contents found by this study.

Table 4 shows that the mean sodium contents of same-category foods vary widely, which is confirmed by the literature^{21,22} and Anvisa^{4,23}. This study used the means obtained by flame photometry, which is more accurate. The variations seen in same-category foods show that it is possible to produce foods with lower sodium contents without affecting the technology used for their production^{4,7,24}.

Foods with colorful packaging and popular characters, drawings and games attract the young public and indicate to adults that they are meant for children. However, this is not the case since the mean sodium contents of these products quantified by this study are higher than those found in similar products by Anvisa^{4,7}.

Moreover, Table 2 shows that, according to flame photometry, all study products contained more than 480mg of sodium per serving, so they are considered high-sodium foods¹⁸. It is also noteworthy that the serving sizes are not necessarily equal to the consumed portion sizes and the meal may have other high-sodium foods, elevating sodium ingestion and significantly contributing to an excessive sodium intake by children and adolescents.

Given the high intake of processed foods by children and adolescents²⁵⁻²⁸, the replacement of meals by snacks^{24,29} and the high sodium contents of the study foods, one can infer that they contribute to a high sodium intake.

The existence of foods that provide as much as 100% of the UL for sodium, such as some instant noodles, shows that sodium content exceeds reasonable parameters and, as intake exceeds the UL, the associated health hazards also increase³⁰.

The risk factors for CNCD are widespread in society. People are often exposed to them early and/or during most of their lifetime³. A study assessed the introduction of processed foods to infants in public daycare centers of *São Paulo* and found that instant noodles are offered to 77.8% of the children aged 12 months or less³¹. Another recent study with 1,131 students aged 7 to 14 years found a prevalence of pre-hypertension of 9.6% and of hypertension of 4.8%; the prevalences were greater in females, individuals with excess weight and individuals with inappropriate diets³².

Since the consumption of processed foods by children and adolescents increases because of women working outside the home, greater convenience, quick preparation, durability and acceptance²⁶, peer influence, higher purchase power, habit of eating out²⁷, and advertising and marketing^{25,28}, asking the food industry to reduce sodium content of their products is considered more effective than asking people to reduce the consumption of these products³³.

This reduction in the sodium content of processed foods will be achieved according to the goals established by the MH and the food industry for the coming years. However, given the differences between the reported and quantified sodium contents, monitoring will be necessary to ensure industry compliance with these goals. This agreement, along with other health-promoting actions, will greatly contribute to the prevention and reduction of CNCD, including hypertension³.

Since some study products were not in compliance with the legislation, better surveillance is necessary. Given that the pertinent laws have been around for a number of years, it is unacceptable that packaging still contains misleading and incorrect information, that products do not comply with the defined identity and amount, and that compulsory nutrition

information is manipulated to become one more marketing strategy.

The technical regulation that establishes the minimum requirements for the supply, marketing, advertising, information and other correlated practices that aim to market foods with high sugar, saturated fat, trans fat and sodium contents, and beverages with low nutrient contents, do not apply to food labeling²³, so this important marketing vehicle continues to be available to the food industry.

CONCLUSION

The results show that the study foods contain high sodium contents that must be reduced. The government's goal to reduce sodium intake and its partnership with the food industry to reduce the sodium content of many foods are essential initiatives but not enough. Encouraging the consumption of fresh foods and providing effective education measures are critical for the promotion of healthy eating habits and minimization of the prevalence of CNDC.

CONTRIBUTORS

VF RIBEIRO responsible for data collection and analysis and article writing. MA RIBEIRO responsible for project creation and article review. MAS VASCONCELOS responsible for reviewing data analysis and the article. SAC ANDRADE responsible for the statistical analysis of the data. TLM STAMFORD co-responsible for project creation and article review.

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Percepção dos cozinheiros escolares sobre o processo de utilização de produtos orgânicos na alimentação escolar em municípios catarinenses

School lunch cooks' perception of the use of organic foods in the school meals served in Santa Catarina state, Southern Brazil

David Alejandro GONZÁLEZ-CHICA¹
Arlete Catarina Títtoni CORSO¹
Francieli CEMBRANEL¹
Kátia Jakovljevic PUDLA¹
Stella LEMKE¹
Bethsáida de Abreu Soares SCHMITZ¹

RESUMO

Objetivo

Identificar o perfil dos cozinheiros escolares e avaliar a utilização, os benefícios e os possíveis fatores limitantes da introdução de alimentos orgânicos na alimentação escolar.

Métodos

Estudo transversal e exploratório incluindo os cozinheiros chefes de 242 escolas públicas municipais dos 52 municípios do estado de Santa Catarina que compraram alimentos orgânicos para a alimentação escolar em 2010. Os entrevistados foram questionados sobre a utilização de alimentos orgânicos, seus benefícios, as dificuldades e a capacitação recebida sobre esse tema. As prevalências e os IC95% foram calculados no *software* Stata 11.0.

Resultados

Dos 242 cozinheiros entrevistados, 97,4% eram do sexo feminino, 86,6% apresentavam 30 ou mais anos de idade e 47,9% não cursaram o ensino médio ou superior. Entre esses, 9,4% (IC95% 6,1-13,9) desconheciam que a escola estava recebendo alimentos orgânicos. Entre aqueles que referiram usar alimentos orgânicos

¹ Universidade Federal de Santa Catarina, Departamento de Nutrição, Programa de Pós-Graduação em Nutrição. Campus Universitário, Trindade, 88040-900, Florianópolis, SC, Brasil. Correspondência para/Correspondence to: DA GONZÁLEZ-CHICA. E-mail: <david.epidemiologia@gmail.com>.

(n=219), 42,7% (IC95% 34,3-51,5) referiram presença diária desses alimentos no cardápio escolar. A qualidade dos alimentos no momento da entrega na escola foi considerada ótima/boa em 93,7% dos casos. Quase 10,0% relataram dificuldades no uso desses alimentos, especialmente por problemas no recebimento/armazenamento e baixa aceitação pelos alunos. A maioria percebeu benefícios no uso de orgânicos na alimentação escolar, tanto para os alunos (99,8%) quanto para a comunidade (100,0%).

Conclusão

O percentual de dificuldades encontrado pelos cozinheiros no uso dos alimentos orgânicos foi baixo, sendo positiva a avaliação quanto ao rendimento, a durabilidade, a quantidade de trabalho e a qualidade desses produtos em comparação aos não orgânicos. Os possíveis fatores limitantes poderiam ser corrigidos mediante melhoria na estrutura física das escolas, na logística de recebimento/estocagem desses alimentos, na capacitação direcionada dos cozinheiros e na sua maior integração nas políticas de alimentação escolar.

Termos de indexação: Alimentação escolar. Alimentos orgânicos. Culinária. Recursos humanos.

ABSTRACT

Objective

This study investigated the profile of school cooks and assessed the use, benefits, and possible limitations associated with the introduction of organic foods in school meals.

Methods

This cross-sectional, exploratory study included the head cooks of 242 public municipal schools of 52 municipalities from the state of Santa Catarina that bought organic foods in 2010. The interviewees were asked about the use of organic foods, their benefits, the associated difficulties, and the training they were given on this subject. The software Stata 11.0 calculated the prevalences and 95% confidence intervals.

Results

Of the 242 interviewed cooks, 97.4% were females, 86.6% were aged 30 or more years, and 47.9% had not completed high school or higher education. Of these, 9.4% (95%CI=6.1-13.9) were not aware that the school was buying organic foods. Of the 219 cooks who used organic foods, 42.7% (95%CI=34.3-51.5) reported that these foods were present every day in the school meals. The quality of these foods upon arrival at the school was considered great/good 93.7% of the time. Almost 10.0% of the interviewees reported difficulties associated with these foods, especially regarding delivery, storage, and low student acceptance. Most interviewees believed that these foods benefited the students (99.8%) and the community (100.0%).

Conclusion

Few cooks had difficulties using organic foods. The cooks found that their yield, shelf life, required work, and quality exceeded those of non-organic foods. The limitations could be eliminated by improving the layout of the schools, food delivery, food storage, cook training, and integration in school food policies.

Indexing terms: School feeding. Food organic. Cooking. Human resources.

INTRODUÇÃO

A alimentação escolar no Brasil é um direito de todo aluno matriculado na educação básica de escolas públicas e filantrópicas, sendo dever do Estado garantir uma alimentação saudável e adequada¹. O Programa Nacional de Alimentação Escolar (PNAE), a partir da Lei nº 11.947, de 16 de junho de 2009, garante o repasse de recursos financeiros e propõe diretrizes com o objetivo de contribuir para o crescimento e o

desenvolvimento dos alunos, para a melhoria do rendimento escolar e para a formação de hábitos alimentares saudáveis^{1,2}.

O emprego da alimentação saudável e adequada compreende o uso de alimentos variados e seguros, que respeitem a cultura, as tradições e os hábitos alimentares dos alunos, sendo recomendado o estímulo ao consumo de gêneros alimentícios diversificados e oriundos de produtores locais, preferencialmente da agricultura

familiar e/ou de empreendedores familiares rurais¹. Por esse motivo, do total de recursos financeiros repassados para o PNAE, no mínimo 30% devem ser utilizados na compra de gêneros provenientes desses produtores, devendo ser priorizada a compra dos alimentos orgânicos³. O cumprimento dessa recomendação é fundamental não apenas por estimular a permanência do pequeno produtor no campo, mas também para potencializar o consumo de frutas e hortaliças desde etapas precoces da vida, favorecendo um maior controle da obesidade e de outras doenças crônicas não transmissíveis, que nas últimas décadas vêm tendo um crescimento acelerado no Brasil^{1,4}.

Por sua vez, o estímulo à utilização de alimentos orgânicos é importante ao se considerar os múltiplos benefícios relacionados ao seu consumo: maior biodisponibilidade de micronutrientes, isenção de agrotóxicos, proteção para os produtores e para o meio ambiente, favorecimento da diversidade biológica e da manutenção da fertilidade dos solos^{5,6}. No entanto, ao longo do processo de utilização dos alimentos orgânicos (desde sua produção até o consumo por parte dos escolares⁷), existem algumas limitações que dificultam sua utilização, tais como o alto preço desses produtos e a falta de cumprimento de exigências higiênico-sanitárias⁸.

Nessa cadeia produção-consumo, os cozinheiros escolares são os executores finais das ações inseridas na política de alimentação escolar, visto que estão diretamente envolvidos na recepção e no preparo de todos os alimentos consumidos pelos alunos. Apesar da relevância dos cozinheiros na alimentação escolar e da relevância da utilização dos alimentos orgânicos, a informação disponível na literatura brasileira sobre a junção desses temas é escassa. Considerando esses aspectos, a finalidade do presente estudo foi identificar o perfil dos cozinheiros escolares, bem como caracterizar questões relacionadas ao uso dos alimentos, aos possíveis fatores limitantes e aos benefícios apontados por eles na utilização

de alimentos orgânicos na alimentação escolar em municípios do estado de Santa Catarina.

MÉTODOS

Foi desenvolvido um estudo transversal e exploratório pelo Centro Colaborador em Alimentação e Nutrição do Escolar de Santa Catarina (CECANE/SC), executado em duas fases. Primeiramente, foi realizado um censo com as Secretarias de Educação de todos os municípios catarinenses, no qual os responsáveis pela alimentação escolar do município informaram sobre a utilização de alimentos provenientes da agricultura familiar, o percentual de recursos destinados para tal objetivo e se o município comprava alimentos orgânicos. O estado de Santa Catarina, localizado no sul do Brasil, está dividido em seis regiões diferentes (Norte, Sul, Oeste, Serrana, Vale do Itajaí e Grande Florianópolis), que incluem um total de 293 municípios. O índice de desenvolvimento humano do estado é 0,84 (segundo lugar no Brasil) e suas principais atividades econômicas são a prestação de serviços, a indústria e o comércio^{9,10}.

Nessa primeira fase da pesquisa, foi utilizado um questionário eletrônico estruturado, o mesmo que foi encaminhado às Secretarias de Educação de todos os 293 municípios do estado de Santa Catarina. O questionário obteve retorno de aproximadamente 90,0% dos municípios (n=264), dos quais 60,6% afirmaram comprar alimentos provenientes da agricultura familiar para a alimentação escolar e 23,1% (n=54) referiram utilizar alimentos orgânicos para tal finalidade, distribuídos da seguinte forma: Norte 7/27, Sul 12/37, Oeste 26/106, Serrana 5/27, Vale do Itajaí 3/48 e Grande Florianópolis 1/19¹¹.

Para a segunda etapa da pesquisa, foi selecionada uma amostra representativa das escolas existentes em todos os municípios que disseram comprar alimentos orgânicos para alimentação escolar em 2009. Inicialmente, foram identificadas as escolas públicas municipais, tanto urbanas como rurais, que ofereceram as modalidades de

ensino infantil e/ou fundamental em 2009. Essa lista foi obtida do censo escolar disponível no *site* do Data Escola Brasil¹², identificando-se 654 escolas atuantes nos 54 municípios que compraram orgânicos em 2009 (média de 12 escolas por município). Com a finalidade de maximizar o número de escolas na amostra, foi usada uma prevalência esperada de 50% para as diferentes informações a serem obtidas, um erro máximo tolerável de 5 pontos percentuais e um alfa de 5%, totalizando uma amostra de 242 escolas. Considerando que todos os municípios que referiram comprar orgânicos seriam elegíveis para a pesquisa (não houve nenhum critério de exclusão no estudo) e que dois dos 54 municípios seriam selecionados para testar o instrumento de coleta de dados, seria necessário entrevistar uma média de cinco escolas por local para assim atingir o tamanho de amostra necessária. Em virtude de 15 municípios (29%) apresentarem um número total de escolas menor que cinco, optou-se por escolher uma amostra de seis escolas nos demais locais. Dessa forma, em municípios com até cinco escolas, foram selecionadas todas as unidades educativas existentes, enquanto entre aqueles com maior número de escolas, foram selecionadas seis instituições, por meio de um processo de amostragem aleatória simples. Essas frações amostrais foram posteriormente consideradas no momento das análises.

Para a execução da segunda fase da pesquisa, foram realizadas no segundo semestre de 2010 visitas *in loco* nos municípios que afirmaram comprar alimentos orgânicos em 2009. As entrevistas foram realizadas com base em questionários estruturados, contendo blocos de perguntas fechadas e abertas em relação a variáveis sociodemográficas dos respondentes, à percepção sobre os benefícios, às dificuldades e às limitações pelo uso de alimentos orgânicos, cursos de capacitação recebidos sobre esse tema, características do processo de recebimento e preparação dos alimentos orgânicos e recomendações para melhoria do processo (questionários disponíveis a partir de solicitação dos autores). As entrevistas foram aplica-

das por quatro entrevistadores com nível superior de educação (três nutricionistas e um agrônomo), os quais foram treinados antes da coleta de dados. Para realizar as entrevistas, cada município foi visitado por um dos entrevistadores entre os meses de setembro e dezembro de 2010, mediante agendamento com a nutricionista da Secretaria de Educação correspondente, ou com o responsável pela alimentação escolar do município, que agendou as visitas com cada uma das escolas selecionadas; visando reduzir a probabilidade de perdas, cada entrevistador permaneceu por até três dias laboráveis em cada município. Em cada uma das escolas, foram entrevistados todos os cozinheiros chefes ou o responsável principal pela preparação dos alimentos na escola.

Para descrição do perfil sociodemográfico dos cozinheiros chefes, foram investigados o sexo (masculino ou feminino), a idade (coletada como variável contínua e categorizada em faixas de 10 anos de idade) e a escolaridade em anos de estudo completos (categorizada posteriormente como 0-4, 5-8, 9-11 e 12 ou mais anos). Em relação ao processo de utilização dos alimentos orgânicos, foram obtidas informações sobre a periodicidade de entrega dos produtos na escola e a periodicidade de inclusão no cardápio dos escolares (diária, semanal, mensal ou anual), a qualidade dos alimentos no momento da entrega (ótima/boa, razoável ou ruim/péssima) e a forma principal de armazenamento (geladeira, câmara fria, despensa ou outra). Os entrevistados também foram questionados sobre as possíveis diferenças encontradas no uso de alimentos orgânicos em comparação aos não orgânicos, considerando quatro variáveis: a durabilidade, o rendimento, a quantidade de trabalho e a quantidade consumida pelos alunos. As quatro variáveis sobre diferenças entre orgânicos e não orgânicos foram categorizadas de forma similar, de acordo com as seguintes categorias: melhorou com os orgânicos, permaneceu igual ou piorou. Os benefícios relacionados com o uso de orgânicos, tanto para os alunos (melhoria da saúde, melhoria no rendimento escolar e maior preocupação com a natureza) quanto para a comunidade (melhoria da

economia regional, aumento da oferta de empregos, proteção do meio ambiente e melhoria da saúde dos produtores), foram coletados como variáveis dicotômicas (não/sim). A mesma categorização foi usada na coleta de informações sobre as dificuldades percebidas com uso dos orgânicos (problemas no recebimento, armazenamento, preparo dos alimentos e aceitação por parte dos alunos) e sobre a participação em cursos de capacitação nos últimos 12 meses (legislação, produção, transporte, armazenamento, preparação e benefícios dos orgânicos).

Os questionários foram digitados diretamente pelos entrevistadores no momento da entrevista em computadores portáteis, mediante formulário digital criado no *software* EpiData 3.1, o mesmo que gerou os bancos de dados correspondentes, os quais foram compactados posteriormente em arquivo único. As análises descritivas foram apresentadas como frequências absolutas e relativas, como os correspondentes Intervalos de Confiança de 95% (IC95%). Em todos os casos, foi considerado o peso amostral das escolas selecionadas (inverso da fração amostral; fração amostral, = número de escolas sorteadas/total de escolas no município), sendo utilizado em todas as análises o conjunto de comandos "survey" (svy) do STATA. As análises estatísticas foram desenvolvidas no *software* Stata 11.0 (Stata Corp., College Station, Estados Unidos).

Todos os participantes da pesquisa assinaram um Termo de Consentimento Livre e Esclarecido no momento da entrevista. O projeto de pesquisa foi aprovado em 31/08/2009 pelo Comitê de Ética em Pesquisa com Seres Humanos da Universidade Federal de Santa Catarina (Parecer nº 1005/2010), de acordo com a Resolução 196/1996 do Conselho Nacional de Saúde do Ministério da Saúde¹³.

RESULTADOS

Nos 52 municípios do estado de Santa Catarina que referiram utilizar alimentos orgânicos na alimentação escolar, a taxa de resposta nas

escolas selecionadas foi de 100,0%, totalizando 242 cozinheiros escolares. A proporção de escolas visitadas por região foi maior na região Sul (28,2%), seguida das regiões Norte (26,9%), Oeste (23,6%), Serrana (11,8%), Vale do Itajaí (8,3%) e Grande Florianópolis (1,2%). A maioria das escolas pertencia a municípios de pequeno ou médio porte (89,4%), enquanto 10,6% estavam localizadas em municípios de grande porte.

Quanto ao perfil dos entrevistados (Tabela 1), houve predomínio do sexo feminino (97,4%), 86,6% apresentavam 30 ou mais anos de idade e 47,9% não cursaram o ensino médio ou superior.

Do total de entrevistados, 23 cozinheiros e desconheciam que a escola estava recebendo alimentos orgânicos para a alimentação escolar (9,4%; IC95% 6,1-13,9). Entre aqueles que referiram usar alimentos orgânicos na preparação das refeições escolares (n=219; Tabela 2), 78,6% (IC95% 69,7-85,3) indicaram que esses alimentos estiveram presentes no cardápio três ou mais vezes por semana.

A Tabela 2 mostra ainda que a periodicidade de entrega dos alimentos orgânicos na

Tabela 1. Perfil dos cozinheiros chefes (n=242) dos municípios do Estado de Santa Catarina que utilizam alimentos orgânicos na alimentação escolar, 2010.

Autor	n	%	(IC95%)
<i>Sexo</i>			
Feminino	236	97,4	(92,7-99,1)
Masculino	6	2,6	(0,9-7,3)
<i>Idade</i>			
20-29 anos	33	13,5	(9,6-18,6)
30-39 anos	67	27,9	(22,2-33,8)
40-49 anos	77	31,9	(26,0-38,1)
50-59 anos	65	26,8	(21,4-32,9)
<i>Escolaridade*</i>			
0-4 anos	45	18,5	(14,0-24,2)
5-8 anos	81	33,6	(27,7-40,0)
9-11 anos	95	39,4	(33,2-45,9)
≥2 anos	20	8,5	(5,1-12,5)

Nota: *Variável com dados ignorados para uma observação.

IC95%: Intervalos de Confiança de 95%.

escola foi diária ou semanal em 81,5% dos casos (IC95% 74,8-86,7). A qualidade dos produtos orgânicos entregues nas escolas foi apontada como boa/ótima por mais de 90,0% dos cozinheiros, e em quase 75,0% dos casos as principais formas de armazenamento mencionadas foram a geladeira ou a câmara fria.

Quanto às dificuldades percebidas que limitam o uso dos alimentos orgânicos na alimentação escolar, 10,1% (IC95% 6,4-14,6%) dos entrevistados referiram algum tipo de dificuldade (dados não apresentados em tabelas), sendo que os entrevistados podiam apontar mais de um motivo. As principais dificuldades foram problema de armazenamento desses produtos (7,1%), seguido pela baixa aceitação por parte dos alunos (2,5%) e dificuldades no recebimento (2,5%). A falta de capacitação e a dificuldade de preparo dos alimentos orgânicos foram indicadas como dificuldades por menos de 1,0% dos cozinheiros. Quando questionados sobre sua própria aceita-

bilidade em relação a esses produtos, todos responderam ter boa aceitação.

A Figura 1 apresenta as principais diferenças apontadas pelos cozinheiros que sabiam que a escola recebia esses alimentos no uso de alimentos orgânicos quando comparado ao uso dos não orgânicos (n=219). Tanto para a durabilidade dos alimentos (n=178) quanto para a quantidade de trabalho, 20,0% dos cozinheiros referiram que essas duas situações pioraram com a introdução dos alimentos orgânicos. Por sua vez, quase 65,0% dos cozinheiros referiram melhoria na durabilidade dos alimentos e 75,0% não encontraram diferenças no volume de trabalho. Quase a metade dos entrevistados referiu melhoria no rendimento dos produtos e apenas 5,5% apontaram que essa condição piorou. O item com piores resultados foi o consumo de frutas e vegetais por parte dos alunos, que apresentou redução segundo 58,0% dos entrevistados.

Tabela 2. Uso de alimentos orgânicos na alimentação escolar, segundo a perspectiva dos cozinheiros escolares de municípios do estado de Santa Catarina, 2010.

	n	%	(IC95%)
<i>Frequência no cardápio (n=210)*</i>			
Diária	90	42,7	(34,3-51,5)
3-4 vezes na semana	75	35,9	(35,9-28,1)
1-2 vezes na semana	44	21,2	(14,5-30,0)
Mensal	1	0,2	(0,0-1,7)
<i>Periodicidade de entrega na escola (n=214)*</i>			
Diária	3	1,3	(0,4-3,6)
Semanal	171	80,2	(73,4-85,6)
Mensal	38	17,9	(12,7-24,5)
Anual	2	0,7	(0,2-2,8)
<i>Qualidade quando chegam à escola (n=212)*</i>			
Ótima/Boa	198	93,7	(85,4-97,4)
Razoável	13	6,1	(2,4-14,4)
Ruim/Péssima	1	0,3	(0,0-2,1)
<i>Armazenamento (n=212)*</i>			
Geladeira	153	72,1	(62,4-80,1)
Câmara fria	5	2,5	(0,5-11,2)
Dispensa	44	20,6	(13,3-30,4)
Outras formas de armazenamento	10	4,8	(2,5-8,8)

Nota: *Variável com dados ignorados para uma observação.

IC95%: Intervalos de Confiança de 95%.

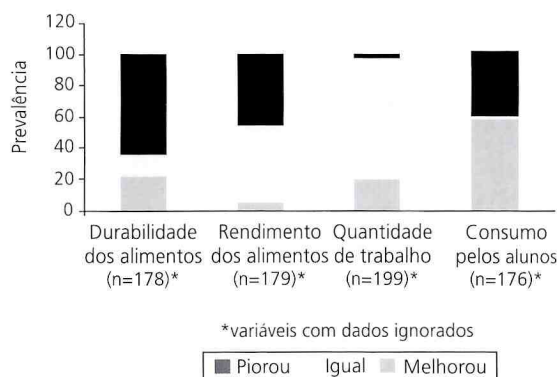


Figura 1. Diferenças encontradas com o uso de alimentos orgânicos em relação aos não orgânicos no preparo da alimentação escolar em municípios do estado de Santa Catarina, 2010. Respostas dos cozinheiros escolares que sabiam que a escola recebia estes alimentos (n=219).

Tabela 3. Percepção dos cozinheiros escolares (n=242) sobre os benefícios relacionados com o uso de alimentos orgânicos na alimentação escolar em municípios do estado de Santa Catarina, 2010.

	n	% de respostas afirmativas
<i>Benefícios para os alunos com uso de alimentos orgânicos</i>	241*	99,8
Melhora saúde em geral	242	100,0
Melhora rendimento escolar	235*	97,0
Maior preocupação com a natureza	235*	97,1
<i>Benefícios para a comunidade com uso de alimentos orgânicos</i>	242	100,0
Melhora economia regional	242	100,0
Aumenta oferta de empregos	238*	98,2
Protege meio ambiente	242	100,0
Melhora saúde de produtores	240*	99,3

Nota: *Variável com dados ignorados.

Ao serem questionados sobre cursos de atualização relacionados ao uso de alimentos orgânicos, e realizados a partir da promulgação da Lei nº 11.947/2009¹, menos da metade dos cozinheiros (42,2%; IC95% 35,7-48,6) afirmou ter realizado algum curso desde aquele ano. Entre aqueles que realizaram esses cursos, o tema de legislação sobre o uso de alimentos orgânicos foi tratado em 58,1% das vezes. Outros temas tratados nesses cursos foram a produção de alimentos orgânicos (71,8%), os cuidados com o trans-

porte e o armazenamento desses produtos (84,2%), os cuidados na preparação (98,6%) e os benefícios para a saúde em geral (95,2%).

A Tabela 3 apresenta a percepção sobre os benefícios que o consumo de alimentos orgânicos na alimentação escolar teria não apenas entre os alunos, mas também na comunidade. Da amostra, todos os cozinheiros referiram que o uso de alimentos orgânicos apresenta benefícios para a saúde dos alunos, e menos de 3% referiram que o uso desses alimentos não aumenta o rendimento escolar e nem a conscientização ambiental. Da mesma forma, todos os entrevistados concordaram quanto ao benefício que o uso desses alimentos traz para a economia regional e para a proteção do meio ambiente, enquanto menos de 2% referiram que o uso desses alimentos não aumenta as fontes de emprego e nem melhora a saúde dos produtores.

DISCUSSÃO

De conformidade com as evidências existentes na literatura científica, esta é a primeira pesquisa realizada no Brasil após a aprovação da Lei nº 11.947/2009 e que teve como finalidade compreender as dificuldades e os benefícios na utilização dos alimentos orgânicos na alimentação escolar, sob a perspectiva dos cozinheiros chefes das cozinhas de escolas municipais. O estudo mostrou que o perfil predominante dos chefes de cozinha foi de indivíduos do sexo feminino, com idade entre 30-49 anos e de baixa escolaridade. O perfil encontrado estaria de acordo com as atividades requeridas para a função¹⁴, que favorece a inclusão de mulheres, sem que para tal função seja necessário um elevado grau de escolaridade. Uma pesquisa realizada em 2009, no município de Santa Fé (PR), por Colombo *et al.*¹⁵, que investigou 16 cozinheiros escolares, mostrou um perfil similar aos resultados do presente estudo quanto ao sexo (todos mulheres) e à escolaridade (mais da metade não havia concluído o ensino fundamental), mas, nesse caso, 80% das participantes

se situavam na faixa etária acima de 50 anos de idade.

O perfil de baixa escolaridade entre os cozinheiros escolares reforça a necessidade de cursos de capacitação para esses profissionais, os quais não apenas deveriam estar direcionados para as boas práticas nutricionais e de controle sanitário, mas também no sentido de favorecer o uso dos alimentos orgânicos². Essa necessidade resulta mais evidente ao se considerar que a Lei 11.947/2009¹ define que, dos recursos da alimentação escolar, 30% devem ser destinados para a compra de produtos da agricultura familiar, com prioridade para a aquisição do gênero dos orgânicos. Mesmo assim, o presente estudo mostrou que menos da metade dos cozinheiros entrevistados afirmaram ter realizado algum curso desde 2009, e menos de dois terços referiram que o tema de legislação foi um dos tópicos abordados durante as capacitações. Associado a isto, vale ressaltar que aproximadamente 10% dos cozinheiros não conheciam que a escola estava recebendo produtos orgânicos para a alimentação escolar. O desconhecimento sobre esta temática por parte dos cozinheiros, assim como a falta de integração sobre as políticas de alimentação escolar que estão sendo executadas, representa um potencial limitante no processo de uso de alimentos orgânicos.

A capacitação insuficiente dos cozinheiros escolares tem sido apontada também por outros estudos. Uma pesquisa realizada em 2005 em Florianópolis (SC) por Lima & Souza⁸ mostrou que nenhum dos cozinheiros escolares participou de programas formais de capacitação. Porém, o mesmo estudo mostrou que a realização de visitas aos fornecedores da alimentação orgânica foi vista como um fator que contribuiu para o conhecimento, a sensibilização e a motivação em relação à temática. Costa *et al.*¹⁶ ressaltam a importância do treinamento com o objetivo de formar profissionais mais críticos, autônomos e capazes para realizar sua função com competência. Carvalho *et al.*¹⁷, em pesquisa desenvolvida na Paraíba

em 2005, destacam também a importância da formação de cozinheiros e revelam ausência de treinamentos sistemáticos e capacitação desses profissionais, sendo citada apenas a realização de cursos ou de palestras esporádicas. O mesmo estudo aponta que os cozinheiros consideram seu trabalho desvalorizado e parecem ter suas atividades norteadas por um conhecimento empírico adquirido por anos de prática. Leite *et al.*¹⁸, em estudo realizado no ano de 2007, em Salvador (BA), sobre uma proposta metodológica para formação de cozinheiros de escolas, revelaram que a realização periódica de cursos de capacitação foi sugerida pelos funcionários como uma estratégia benéfica para a realização das suas atividades. Dessa forma, a promoção de políticas de capacitação, valorização e integração desses funcionários pode constituir uma alternativa para melhorar o trabalho realizado e promover uma alimentação adequada aos alunos.

Em relação à utilização dos alimentos orgânicos, aproximadamente 40% dos entrevistados referiu que eles são incluídos no cardápio dos escolares com periodicidade diária, e em quase 80% das escolas a frequência foi de três ou mais vezes por semana. Embora não existam dados prévios à promulgação da Lei 11.947/2009¹, os nossos dados sugerem que houve avanços na inserção dos orgânicos na alimentação escolar, principalmente entre municípios de pequeno e médio porte. Em municípios de grande porte, no Brasil, foi localizada apenas uma pesquisa sobre este tema, realizada em 2005 em uma escola de ensino fundamental de Florianópolis (SC), que mostrou que os alimentos orgânicos estiveram presentes no cardápio escolar somente em 7 dos 21 dias avaliados⁸. Países como Itália, Finlândia, Dinamarca e Noruega mostram que é possível aumentar a cobertura e a frequência dos alimentos orgânicos oferecidos aos escolares¹⁹.

Chaves *et al.*²⁰ analisaram a presença de alimentos regionais nos cardápios nas diversas regiões do País, e apontaram a Região Sul como a que, proporcionalmente, apresentou mais preparações regionais (86,5%) comparada ao restan-

te do país, que oscilou entre 38% (Norte) e 84% (Norte e Sudeste). Embora esses autores não tenham avaliado a presença de orgânicos na alimentação escolar, esse achado reforça a necessidade da preocupação da inclusão desses itens na alimentação dos escolares.

Os autores Lima & Souza⁸ relatam como fatores limitantes para a utilização de alimentos orgânicos na alimentação escolar o pequeno número de fornecedores certificados e o alto preço dos alimentos orgânicos, bem como problemas referentes à pontualidade da entrega, à solicitação e ao atendimento das compras, assim como à falta de cumprimento aos critérios higiênico-sanitários da embalagem e à rotulagem dos produtos. No presente estudo, o processo de distribuição dos alimentos orgânicos e a sua qualidade não foram limitantes para sua utilização. Apenas 2,5% dos cozinheiros chefes apontaram problemas no recebimento desses produtos, e, na maioria dos casos, eles foram classificados como de qualidade boa ou ótima. Assim, aparentemente, houve melhorias no processo de distribuição dos produtos após a Lei nº 11.947/2009¹, o que poderia estar relacionado com o incremento no número de produtores rurais¹¹.

Outro possível limitante no processo de distribuição seria a falta de estrutura física apropriada nas escolas para receber e armazenar esses produtos, que foi relatado por 10% dos entrevistados. Outros países que também oferecem alimentos orgânicos nas escolas precisaram encontrar diversos caminhos para superar essas dificuldades. Na Finlândia, por exemplo, na década de 1970, foram realizados grandes investimentos para melhorar a infraestrutura das cozinhas. Já em países como a Dinamarca e a Noruega, a solução aos problemas relacionados com a estrutura física das cozinhas foi a oferta de alimentos orgânicos embalados, como frutas e leite, embora exista a iniciativa de oferecer alimentos cozidos¹⁹.

De acordo com as respostas dos cozinheiros, outro fator relevante é a baixa aceitação

por parte dos alunos. Mesmo que 2,5% dos entrevistados tenham apontado este aspecto como uma dificuldade, em mais da metade dos casos os cozinheiros perceberam piora no consumo de frutas e vegetais quando os alimentos orgânicos foram introduzidos no cardápio. Vários fatores podem afetar o consumo dos alimentos oferecidos para os escolares neste programa, como características da família e da própria escola²¹. Embora as causas subjacentes para o menor consumo de alimentos orgânicos por parte dos escolares não tenham sido investigadas no presente estudo, a ação dos cozinheiros não parece ser um fator limitante: referiram ter capacitação suficiente para preparar esses alimentos, consideram benéfico o uso desses alimentos e não encontraram dificuldades na sua preparação. A durabilidade e o rendimento dos produtos foram apropriados, e não houve aumento na carga de trabalho. O estudo de Lima & Souza⁸, realizado em 2005, mostrou também que os cozinheiros escolares não observaram diferenças quanto ao rendimento, à aparência e ao tempo de vida útil entre alimentos orgânicos e não orgânicos. Essas e outras qualidades dos alimentos orgânicos têm sido amplamente investigadas: em vários aspectos, esses produtos apresentam melhores características nutricionais do que os não orgânicos^{6,22}.

Vale destacar que o uso de alimentos orgânicos na alimentação escolar vem de acordo com a Política Nacional de Segurança Alimentar e Nutricional (PNSAN), cuja proposta é regida pelos parâmetros do Sistema Nacional de Segurança Alimentar e Nutricional (SISAN) de acordo com a Lei nº 11.346/2006²³. A política assegura uma alimentação adequada a toda população com práticas sustentáveis que respeitem o ambiente, a cultura, a economia e a sociedade²⁴. Nesse sentido, o uso de alimentos orgânicos, especialmente de pequenos produtores e advindos da agricultura familiar, é uma alternativa que beneficia as crianças, os pequenos produtores e o meio ambiente.

Embora o presente estudo apresente como limitação a avaliação de uma parte dos municípios

no Estado de Santa Catarina, foram nele incluídos todos aqueles que compraram alimentos orgânicos em 2010. Da mesma forma, foi selecionada uma amostra representativa das escolas, e a taxa de resposta foi de 100%. Assim, os resultados do presente estudo poderiam refletir a situação de outros municípios que estejam em processo de usar produtos orgânicos na alimentação escolar.

CONCLUSÃO

Os cozinheiros escolares desempenham um papel central na utilização de produtos orgânicos na alimentação escolar. Apesar da boa percepção e da capacitação dos cozinheiros sobre os benefícios e a facilidade na utilização dos desses alimentos, algumas dificuldades foram citadas, envolvendo principalmente os processos de recebimento e estocagem desses produtos. Esses problemas poderiam ser corrigidos mediante melhoria na infraestrutura das cozinhas e de cursos de capacitação direcionados a melhorar o recebimento e a estocagem dos gêneros orgânicos. Por sua vez, novos estudos precisariam ser realizados para investigar os motivos subjacentes para a redução no consumo de frutas e verduras que aconteceu com a introdução dos alimentos orgânicos, situação que foi apontada por mais da metade dos cozinheiros.

Os resultados apresentados fornecem dados importantes que podem ajudar na inclusão dos alimentos orgânicos provenientes da agricultura familiar na alimentação escolar em todas as escolas do estado de Santa Catarina e apontam para a necessidade de ações governamentais conjuntas que procurem articular todos os atores envolvidos neste processo e que valorizem a utilização desses produtos na alimentação escolar.

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Quail egg safety and trade on beaches of Salvador (BA): A study from a child labor perspective¹

O comércio e a segurança de ovos de codorna em praias de Salvador (BA): um estudo na perspectiva do trabalho infantil

Perminio Oliveira VIDAL JÚNIOR²
Ryzia de Cassia Vieira CARDOSO³
Larissa Santos ASSUNÇÃO⁴

ABSTRACT

Objective

This study aimed to describe the trade and microbiological quality of boiled quail eggs on the waterfront of Salvador, Bahia, Brazil, from the child labor perspective.

Methods

This cross-sectional study administered semi-structured questionnaires to 40 underage vendors and performed the microbiological assessment of 40 quail egg samples as follows: mesophilic aerobic microorganism count, coagulase-positive staphylococcus count, estimation of the most probable number of total and thermotolerant coliforms/*Escherichia coli*, and testing for *Salmonella* spp.. The results were compared with the standards provided by the Resolution RDC n° 12/2001, National Sanitary Surveillance Agency.

Results

The vendors were mostly female (57.5%) students (95.0%) aged 8 to 17 years. The most common reason for working was supplementation of the family income (57.5%). The mean gross income was R\$38.31/day. Most of them presented inadequate personal hygiene but they recognized that foods could cause diseases. Many (47.5%) vendors reported washing their hands up to twice daily. Mean mesophilic aerobic microorganism and coagulase-positive staphylococcus counts were 2.43 and 2.01 log colony-forming unit/g, respectively, and the

¹ Article based on the dissertation of PO VIDAL JÚNIOR, intitled "Comida de rua e segurança de alimentos na orla marítima de Salvador-BA: um estudo na perspectiva do trabalho infantil". Universidade Federal da Bahia; 2011.

² Universidade Federal do Recôncavo da Bahia, Centro de Ciências da Saúde, Curso de Nutrição. R. do Cajueiro, s/n., Cajueiro, 44574-490, Santo Antônio de Jesus, BA, Brasil. *Correspondência para/Correspondence to:* PO VIDAL JÚNIOR. *E-mail:* <perminiojr@ufrb.edu.br>.

³ Universidade Federal da Bahia, Escola de Nutrição, Departamento de Ciências dos Alimentos. Salvador, BA, Brasil.

⁴ Academic, Universidade Federal da Bahia, Escola de Nutrição. Salvador, BA, Brasil.

estimated thermotolerant coliform contamination was 0.98 log most probable number/g. *Escherichia coli* was found in 15.0% of the samples and none contained *Salmonella spp.* Most (55.0%) samples were noncompliant with the legislation.

Conclusion

The results evidenced the presence of minors selling quail eggs on beaches of Salvador and suggest risk to consumers' health because of the detected contamination and vendors' ignorance of principles of hygiene.

Indexing terms: Child labor. Eggs. Street food.

RESUMO

Objetivo

Este estudo buscou caracterizar o comércio e a qualidade microbiológica de ovos de codorna cozidos, na orla de Salvador, Bahia, na perspectiva do trabalho infantil.

Métodos

*Realizou-se estudo transversal, com aplicação de questionários semi-estruturados, junto a 40 vendedores menores de idade, e análise microbiológica de 40 amostras, submetidas aos procedimentos que seguem: contagem de micro-organismos aeróbios mesófilos, estafilococos coagulase-positiva e estimativa do número mais provável de coliformes totais e termotolerantes/*Escherichia coli* e pesquisa de *Salmonella spp.* Os resultados foram comparados com padrões da Resolução RDC n° 12/2001, Agência Nacional de Vigilância Sanitária.*

Resultados

*Os vendedores eram predominantemente meninas (57,5%), tinham faixa etária entre 8 e 17 anos e estudavam (95,0%). A complementação da renda familiar foi a razão mais apontada para o trabalho (57,5%), sendo a renda média na atividade de R\$38,31/dia. A maioria não observava requisitos de higiene pessoal, porém considerou que os alimentos poderiam veicular doenças. Quanto à frequência de lavagem das mãos, a maioria (47,5%) declarou lavá-las até duas vezes/dia. As contagens de micro-organismos aeróbios mesófilos e estafilococos coagulase-positiva registraram valores médios de 2,43 e 2,01 log unidade formadora colônia/g, respectivamente, enquanto a estimativa de termotolerantes foi de 0,98 log número mais provável/g; identificou-se *Escherichia coli* em 15,0% das amostras e ausência de *Salmonella spp.* Entre as amostras, 55,0% classificaram-se como não conformes.*

Conclusão

Os resultados evidenciam a inclusão de menores de idade no comércio de ovos de codorna, nas praias e sugerem riscos aos consumidores, devido à contaminação registrada e ao desconhecimento dos vendedores quanto aos princípios de higiene.

Termos de indexação: Trabalho de menores. Ovos. Comida de rua

INTRODUCTION

Consumption of street food is an old practice that has been growing in the last decades, especially because of socioeconomic changes. This segment offers a diversity of foods and beverages for immediate or later consumption. This type of trade has a high economic potential and its development aims to meet the needs of part of the population who requires fast and cheap meals, preserving food traditions¹⁻³.

In this scenario, street food trade increases employment and provides income to less

fortunate social groups, markedly including people of working age, although this segment also represents a work reality of children and adolescents^{4,5}.

In general, based on how its trade is conducted, street food is a risk to consumers' health, especially because of the absence of a minimum structure that allows the use of hygiene procedures. Moreover, other factors, such as poorly trained vendors, inappropriate hygiene habits and inadequate knowledge on food handling techniques, have been reported in studies with packmen⁶⁻⁸.

In this complex picture, while this trade provides employment, income and better quality of life to part of the population, it may also make people fall victims to their ignorance of proper food handling practices, since foods may carry many pathogens^{1,9}. This is a special concern with regard to children and adolescents, since underage vendors usually do not know how to properly handle and conserve foods, so they represent a source of possibly unsafe foods for the population.

On the beaches of *Salvador* (BA) and metropolitan region, it is common to see children and adolescents selling foods and beverages, including homemade and processed foods. Among these, boiled quail eggs sold in their shells stand out, since they are much appreciated for their taste, nutritional value and aphrodisiac properties¹⁰, and are easy to carry by underage vendors.

As indicated by some data from the Instituto Brasileiro de Geografia e Estatística¹¹, the national production and consumption of quail eggs has been growing, and the main producers are the Brazilian Southeast and the states of *Pernambuco* and *Bahia*. Raw eggs are perishable and susceptible to changes that affect its quality. The rate of these changes depends on storage temperature and conditions¹².

Generally, raw eggs are contaminated after they are laid, with little or no contamination during oviposition¹³⁻¹⁵. Possible contamination routes include transovarian contamination and direct contact with bird feces, which facilitate the entrance of microorganisms through the shell pores, limiting the number of processes that are capable of disinfecting raw eggs¹⁵.

When shelled eggs are boiled, they require additional care, since this thermal treatment destroys the protective membrane that lines the shell and exposes the pores to bacteria that may contaminate them. Hence, for better safety and preservation, home-boiled eggs should be refrigerated within two hours of boiling and consumed within one week¹⁶.

Given the above, this study aimed to characterize the trade and microbiological safety of home-boiled, shelled quail eggs sold on the waterfront of *Salvador* (BA) from the child labor perspective.

METHODS

This descriptive, cross-sectional study was done between January and October of 2010 on the waterfront of *Salvador* as part of the project "*Comida de rua e trabalho infantil: o descortinar de uma realidade na orla marítima de Salvador (BA) e a busca da segurança alimentar e da inclusão social*" (Street food and child labor: unveiling a reality on the waterfront of *Salvador* (BA) and the quest for food safety and social inclusion).

Calculation of the sample size of the abovementioned project was based on a child labor prevalence of 8.7%, which includes all forms of labor of 5-to-17-year-olds in the Metropolitan Region of *Salvador* (BA)¹⁷. An error of 3.0% and critical α of 0.05 were used in the calculation, resulting in a sample size of 340 vendors.

A preliminary study identified the most common foods sold by underage vendors on 18 beaches. Given the absence of information necessary for the calculation of a probabilistic sample of quail egg vendors, a non-probabilistic subsample of 40 vendors was established for data and sample collection. Accidental sampling¹⁸ was used in eight waterfront locations - *São Tomé de Paripe, Ribeira, Boa Viagem, Canta Galo, Patamares, Jaguaribe, Piatã and Itapuã*. The study was done on weekends, that is, Saturdays and Sundays, during the day.

A semi-structured questionnaire previously tested by a pilot study was used for collecting information about the trade and vendors. The questionnaire was divided into four sections: vendor socioeconomic characteristics; food profile, acquisition and storage; hygiene and sanitary characteristics of the vendor and work; and vendor opinion.

The questionnaire was filled out by trained, supervised interviewers who collected information by direct observation and interview, depending on the question.

A sample of 15 boiled quail eggs, enough to compose an analytical unit, was purchased from each vendor for assessment of their microbiological profile. A total of 40 samples were acquired. The samples were collected aseptically, placed in sterile plastic bags which were then placed in coolers with ice and sent to the Laboratory of Food Quality Control of *Universidade Federal da Bahia* (UFBA) School of Nutrition. The samples remained under refrigeration for a maximum of four hours until analysis.

Microbiological analyses of the quail eggs consisted of Mesophilic Aerobic Microorganism (MAM) count, Coagulase-Positive Staphylococcus (CPS) count, Most Probable Number (MPN) of total and thermotolerant coliforms/*Escherichia coli*, and testing for *Salmonella spp.* (SAL). The procedures were done as recommended by the American Public Health Association (APHA)¹⁹.

In order to analyze the external and internal contamination of the quail eggs as suggested by the International Organization for Standardization (ISO) 6887-4²⁰, the broken shells and eggs were soaked together in the collection bags. This procedure was chosen because boiled quail eggs are handled by vendors and consumers, who then peel the eggs with bare hands and transfer microbes from the shells and hands to the egg, which is then eaten.

The results of the microbiological analyses were compared with the limits established by Resolution RDC nº 12/2001²¹, issued by the *Agência Nacional de Vigilância Sanitária* (Anvisa, National Sanitary Surveillance Agency). The study food was classified as a ready-to-eat dish (ready-to-eat foods prepared in home kitchens, restaurants and similar establishments), category A, which includes meat-based foods, boiled eggs and similar foods. This classification was based on the similar nature and processing of the product.

The data collected by the questionnaires were entered in a database and analyzed by the software Statistical Package for the Social Sciences (SPSS), version 20.0. The study variables were analyzed descriptively and the Pearson's chi-square test was used for investigating associations of interest among the variables. The significance level was set at 5% ($p < 0.05$). The prevalence of samples contaminated by indicator and pathogenic microorganisms was also calculated by dividing the number of samples not complying with the legislation by the total number of samples.

As required by Resolution nº 196/96²² issued by the National Health Council, the study was approved by the Research Ethics Committee of the UFBA *Hospital e Maternidade Clímério de Oliveira*, located in *Salvador* (BA), under protocol number 14/2008. The interviews were done after the vendors' guardians signed a free and informed consent form or, if the guardians were not around, when vendors agreed to participate in the study.

RESULTS

Vendor identification and socioeconomic characteristics

Most vendors were females and many start selling street food on the waterfront of *Salvador* (BA) before reaching legal working age. Additionally, most children and adolescents interviewed by this study reported attending school regularly, and 72.5% reported that their families were on welfare, confirming their social vulnerability. A small percentage (5.0%) of the older vendors were school dropouts, but vendor age was not significantly associated with education level ($p = 0.26$).

Most vendors did not limit their sales to a single beach and also sold their products elsewhere. The working day of quail egg vendors is long, working an average of 6.6 hours per day, and they remain on the job for a long time, on average, 3.8 years.

Most vendors reported working on weekends to take advantage of the greater number of consumers on beaches and/or the days off school, although some vendors admitted to working during schooldays. Because of the long working day, most children and adolescents worked two shifts during the day.

Table 1 shows that vendors are either self-employed or have different employers: their families or someone close to their homes (relatives or neighbors). They have a mean gross income of R\$38.31 *per day*. A complementary test that investigated the association between age and employer showed that, while adolescents were self-employed, children generally worked for their parents or third parties ($p=0.03$). Finally, adolescent quail egg vendors were more prevalent than child vendors, regardless of gender.

Supplementation of the family income was the most common reason for participants to become street food vendors (57.5%). Other reasons included the need of being financially self-sufficient (22.5%), having an occupation (17.5%) and turning their work into their main income means (2.5%).

Quail egg profile, acquisition and storage

In general, quail eggs sold on beaches were bought at a large farmer's market in *Salvador* (BA), called *São Joaquim*, or occasionally, in people's markets. The eggs were prepared at home by the vendors' mothers (57.5%), other family members (20.0%), neighbors (12.5%) or the vendors themselves (10.0%). After boiled, the eggs were returned to the original packaging (egg carton) and taken to the point of purchase.

On the beaches, 85% of the vendors piled the egg cartons, with 30 eggs each, on their arms, and 15% used plastic or cardboard boxes to carry the egg cartons and salt for seasoning, according to the customers' requests.

Hygiene and sanitary characteristics

Figure 1 shows the quail egg vendors' compliance with hygiene requirements. It is evident that compliance with hygiene criteria was inadequate, which adds to the age incompatibility of the vendors with the activities related to informal food trade.

The majority of the vendors reported washing their hands up to twice daily (47.5%), from two to five times daily (27.5%) and more than five times daily (12.5%). A few (12.5%) reported not washing their hands during their

Table 1. Demographic, socioeconomic and work characteristics of street food vendors (n=40) who sell boiled quail eggs on the beaches of *Salvador* (BA), Brazil, 2010.

Characteristics	Distribution	
<i>Gender (%)</i>		
Female	57.50	(23)
<i>Age</i>		
Mean (amplitude)	13.30	(8 - 17)
<i>School attendance (%)</i>		
Yes	95.00	(38)
<i>Work location (%)</i>		
Only one beach	37.50	(15)
More than one beach	57.50	(23)
Other locations	5.00	(2)
<i>Length of employment (years)</i>		
Mean (amplitude)	3.80	(0.08 - 10)
<i>Working day (hours)</i>		
Mean (amplitude)	6.60	(3 - 13)
<i>Work days (%)</i>		
Saturdays and Sundays	82.50	(33)
Monday to Friday	15.00	(6)
Every day	2.50	(1)
<i>Work shift (%)</i>		
Morning	2.50	(1)
Afternoon	17.50	(7)
All day	80.00	(32)
<i>Employer (%)</i>		
Self	25.00	(10)
Parents	52.50	(21)
Third parties	22.50	(9)
<i>Daily gross income (R\$)</i>		
Mean (amplitude)	38.31	(7 - 80)

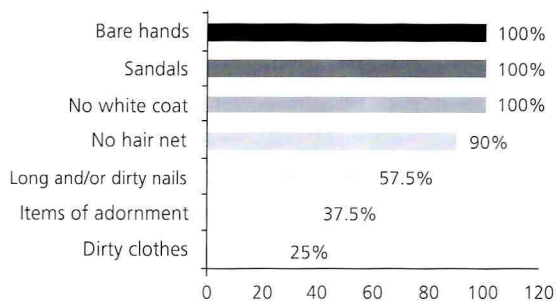


Figure 1. Personal hygiene particularities of children and adolescents (n=40) who sell boiled quail eggs on the waterfront of Salvador (BA), Brazil, 2010.

work shift. Generally, the vendors washed their hands at the water faucets available in beach stands (88.5%) or with seawater (11.5%).

Visual assessment indicated that 12.5% of the vendors had bad or terrible hygiene habits, 75.0% had regular hygiene habits and 12.5% had good hygiene habits.

Vendor perception of good handling practices and their work

When asked about the importance of hygiene in food trade, 97.5% of the children and adolescents considered it important, especially vendor hygiene. In parallel, most (62.5%) believed that street foods could cause diseases in those who consumed them.

Regarding the social aspect of work, 87.5% of the vendors reported enjoying the activity, while the others reported dissatisfaction, sadness or fatigue. On the other hand, when asked about what they wish they could be doing, 77.5% wanted to be doing some leisure activity if they were not busy working.

Microbiological profile of boiled quail eggs

Table 2 shows the results of the microbiological analyses of the boiled quail egg samples. The mesophilic aerobic microorganism counts varied greatly, with 5.0% (n=2) of the samples having counts in excess of 5.0 log Colony-Forming Units per gram (CFU/g).

With respect to total coliforms, 80.0% (n=32) of the samples exceeded 1.3 log MPN/g. Meanwhile, the MPN of thermotolerant coliforms of an expressive percentage of the samples (22.5%) exceeded the limit established by Resolution nº 12/2001²¹. Moreover, *E. coli* was found in 15.0% (n=6) of the samples, confirmed by the Indole, Methyl red, Voges-Proskauer and Citrate tests (IMViC).

Coagulase-Positive Staphylococcus counts exceeded the tolerated limit in 45% (n=18) of the samples and in many others, CPS counts were very close to the tolerated limit. *Salmonella spp.* was not found in any of the samples.

Table 2. Characterization of the boiled quail egg samples (n=40) acquired on the waterfront of Salvador (BA) regarding their microbial contamination (log of the Most Probable Number (MPN) or Colony-Forming Units (CFU/g). 2010.

Microorganisms	Count			RDC nº 12/2001 Standard	Noncompliant* n (%)
	M	SD	Amplitude		
Mesophiles	2.43	1.20	1.30 - 5.51	NA	NA
Total coliforms	1.58	0.86	0.47 - 3.04	NA	NA
Thermotolerant coliforms	0.98	0.98	0.47 - 3.04	1.3	9 (22.5%)
Coagulase-positive staphylococci	2.01	2.00	0.00 - 5.25	3.0	18 (45.0%)
Positive samples n (%)					
<i>Escherichia coli</i>	6 (15.0%)			NA	NA
<i>Salmonella spp.</i>	—			Not found	—
Study samples n (%)					Non-compliant samples
Total	40 (100%)				22 (55%)

Note: *Not in compliance with the current microbiological standards. NA: Not Applicable; M: Mean; SD: Standard Deviation.

The prevalence of samples not complying with the legislation²¹ was considered high (55%), given that the product had been boiled.

According to Pearson's Chi-square test, vendor age and sample noncompliance were not significantly associated.

DISCUSSION

The presence of female street vendors evidenced by the present study is in disagreement with studies²³⁻²⁶ that reported that this kind of work is essentially done by males. In this context, the presence and prevalence of girls selling quail eggs may be justified by the relative ease of the job.

As reported by vendors and their guardians, eggs are light compared with *coalho* cheese and ice pops, which require greater physical effort from packmen. Therefore, the selection of this product may be gender-related, since it requires less physical effort. However, as Acho-Chi²⁷ points out, the presence of girls in informal jobs is more concerning, since there is a correlation between this kind of work and risk of other social problems, such as adolescent pregnancy, early marriage or even child prostitution.

The results indicate that children become street vendors early. In this case, work exposes young packmen to a number of risks, since the required tasks are incompatible with their ages^{8,25}, even if most, especially girls, are supervised by adult family members during their work - a strategy employed by families to minimize job-related risks. According to Schwartzman²⁸, working children aged 5 to 9 years are more prevalent in low-income families.

The long working day and length of employment found by the present study not only make school attendance impossible or unfeasible, but also confirm the relationship between child labor and contribution to family income, that is, children and adolescents stay on the job for very evident economic reasons^{23,29}.

The work characteristics of these vendors unveil a reality that violates the *Estatuto da Criança e do Adolescente* (ECA, Child and Adolescent Statute), and reveal the fragility and vulnerability of the children and adolescents involved in this type of work. In this sense, many risks may be present in their daily routine, such as injuries, long walks in the sun, dehydration, and sexual and/or moral harassment, as pointed out by Carvalho²⁵ and Audu *et al.*³⁰.

The greater participation of adolescents of both genders in this activity probably reflects families' attempts to preserve the younger children, delaying their joining street trade.

Supplementation of the family income as the main justification for becoming a street food vendor may be easily associated with the poverty level of the families involved. However, other determinants may be identified, such as the current economic model that promotes social inequality, market structure, which allows the presence of children and adolescents in the informal sector, and the positive cultural aspect of work³¹⁻³³.

In addition to contributing to the family income, other reasons were mentioned by vendors for having joined the segment, such as having an occupation or becoming financially self-sufficient by selling foods on the city's beaches, which coincide with the reasons reported by Gonçalves *et al.*³⁴ when they described the profile of 14- and 15-year old adolescents in a mid-sized city in the Brazilian South.

This information is also in agreement with Rizzini³⁵, who stated that in this age group, individual factors, such as wanting to have self-earned money, more freedom and an occupation or trade, add to cultural factors, such as the belief that work promotes discipline.

Regarding the preservation of the marketed product, the United States Department of Agriculture (USDA)¹⁶ recommends that boiled eggs should be cooled within two hours of boiling and consumed within one week.

Although the vendors have reported selling all eggs on the day of preparation, this trade requires walking, so the vendors carry their products along city beaches, in the sun for long periods of time and in adverse preservation conditions, promoting the loss of hygienic and sanitary quality of the quail eggs and risks for the consumers.

Furthermore, according to some vendors' reports, often when the eggs were not sold on the same day they were boiled, the vendors would try to sell them on the next day, regardless of the storage conditions, that is, whether they had been refrigerated or not.

The results that characterize the hygiene of the young vendors reflect their inability, when compared with adult vendors, to meet food trade requirements. However, even adult food vendors who work in the informal sector have been blamed of unsatisfactory hygiene practices by Mallon & Bortolozzo³⁶ in a study of adult packmen from *Paraná*, and by Omemu & Aderoju⁹ and Umoh & Odoba³⁷ in studies that assessed the hygiene practices of street food vendors in Africa.

Other studies have found more concerning results³⁷⁻³⁹, such as vendors with inadequate hygiene habits and working in inappropriate conditions.

Considering the microbiological profile of the study samples, although the legislation does not establish a limit for mesophiles, their count indicates the general contamination of a food and its hygiene quality. Hence, their counts allow the assessment of the hygiene and preservation status of a food^{19,40}.

Although the mean mesophile count found by the present study was not high, two samples (5%) contained counts in excess of 5.0 log CFU/g, which may suggest inappropriate handling, processing and/or storage, and/or exposure of the product to adverse environmental conditions⁴⁰.

Likewise, there are no standards for total coliforms but the comparison of the study counts

for this group of microorganisms with the specific standard for MPN of thermotolerant coliforms per gram may be useful for indicating inadequate hygiene after processing⁴¹. Given that 80.0% (n=32) of the samples had high total coliform counts and that 22.5% (n=9) exceeded the limit for thermotolerant coliforms established by the legislation²¹, the results are concerning, especially when one considers that boiling kills many microorganisms.

Additionally, the high coagulase-positive staphylococcus count found in 45.0% of the samples indicates inappropriate storage and handling, since humans are one of the main reservoirs of this microorganism⁴². This statement is confirmed by the field findings, that is, many vendors were not careful when handling the product and maintained the product under inappropriate temperature and storage conditions for long periods of time.

This finding is similar to one found by Umoh & Odoba³⁷ when they investigated the quality of street food sold in Zaria, Nigeria, considering the pertinent proportions, since the latter assessed different foods. The literature on quail eggs focuses on fresh quail eggs and the profile of their consumption and consumers. Thus, the scarcity of information on the quality of boiled quail eggs prevented the direct comparison of the results of the present study with other studies.

On the other hand, even though contamination by *Salmonella spp.* has not been found in the study samples, the counts of indicator microorganisms suggest that better handling practices are necessary, especially after the product is boiled, since boiling destroys the protective membrane that lines the shell, facilitating microorganism access to the egg white and yolk¹⁶.

Few of the vendors reported cleaning the egg cartons used for transporting the boiled eggs. Hence, not cleaning the egg cartons or not cleaning them properly may partly justify the

microorganism counts, since the boiled eggs may be suffering cross-contamination.

More importantly, consumers believe that boiled eggs inside their shells are safe. However, when they handle and peel the eggs with unwashed hands, they not only contaminate the eggs with the microorganisms present on their hands, but also with those present on the shells, increasing the microbial load of the food.

CONCLUSION

The objective of this study was to characterize the trade and describe the microbiological quality of boiled quail eggs sold on the waterfront of *Salvador* (BA) from the child labor and food safety perspective. In addition to confirming the presence of minors selling street foods, the study results also point out the social risks associated with the work routine of the children and adolescents involved.

What is more, the expressive prevalence of noncompliant samples indicates that the consumption of quail eggs on beaches poses a health risk. Therefore, the inept labor of the vendors combined with unfavorable work conditions may have contributed to the study results.

Given the nature and complexity of child labor and the problems associated with informal food trade, strategies are needed to protect consumers' health and the children and adolescents who work as street food vendors, and to ensure the quality of the foods sold.

CONTRIBUTORS

VIDAL JÚNIOR PO collected, analyzed and interpreted the data and wrote the article. CARDOSO RCV participated in data analysis and interpretation, and article writing and review. ASSUNÇÃO LS collected, analyzed and interpreted the data and helped to write the article.

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Diet quality in a sample of adults from Cuiabá (MT), Brazil: Association with sociodemographic factors

Qualidade da dieta de uma amostra de adultos de Cuiabá (MT): associação com fatores sociodemográficos

Anarlete da Silva LOUREIRO¹

Regina Maria Veras Gonçalves da SILVA²

Paulo Rogério Melo RODRIGUES³

Rosângela Alves PEREIRA³

Loiva Lide WENDPAP²

Márcia Gonçalves FERREIRA²

ABSTRACT

Objective

To analyze the dietary quality of adults and to identify associated factors.

Methods

Cross-sectional study carried out in a sample of adults (n=195), aged 20-50 years, of both genders, from a population-based study in *Cuiabá, Mato Grosso, Brazil*. Food consumption data was collected by a food frequency questionnaire. Sociodemographic, lifestyle, anthropometric, and body composition data were also collected. Diet quality was analyzed by the Brazilian Healthy Eating Index-Revised. The associations were estimated by Poisson regression.

Results

The mean Brazilian Healthy Eating Index-Revised score was 75.2 points (CI95%=74.2-76.1), which differed significantly between the genders ($p=0.03$). Women had higher scores for whole fruit and sodium ($p<0.01$),

¹ Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso. *Campus Cáceres*, Av. dos Ramires, s/n., Distrito Industrial, 78200-000, Cáceres, MT, Brasil. *Correspondência para/Correspondence to:* AS LOUREIRO. *E-mail:* <anarlete.loureiro@cas.ifmt.edu.br>.

² Universidade Federal de Mato Grosso, Faculdade de Nutrição, Departamento de Alimentos e Nutrição. Cuiabá, MT, Brasil.

³ Universidade Federal do Rio de Janeiro, Instituto de Nutrição Josué de Castro, Departamento de Nutrição Social e Aplicada. Rio de Janeiro, RJ, Brasil.

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while men had higher scores for oils, nuts, and fish fat ($p=0.02$). Individuals aged ≥ 30 years had higher total Brazilian Healthy Eating Index-Revised score and higher scores for the groups whole fruit; saturated fat; and calories from solid fats, alcoholic beverages, and added sugar ($p<0.01$). The Poisson regression between high Brazilian Healthy Eating Index-Revised and the independent variables showed that high Brazilian Healthy Eating Index-Revised was associated with being female, being aged 30 years or more, and being from families whose household head had 8 or more years of formal education.

Conclusion

The factors associated with high Brazilian Healthy Eating Index-Revised were age, gender, and education level of the household head.

Indexing terms: Adult. Diet. Food consumption.

RESUMO

Objetivo

Analisar a qualidade da dieta e identificar fatores associados em adultos residentes na área urbana de Cuiabá, Mato Grosso, Brasil.

Métodos

Estudo transversal com amostra de 195 adultos de 20 a 50 anos, de ambos os sexos, extraída de estudo de base populacional. A ingestão alimentar foi avaliada por questionário de frequência de consumo alimentar. Foram coletados dados sociodemográficos, de estilo de vida, antropométricos e de composição corporal. A qualidade da dieta foi analisada pelo Índice de Qualidade da Dieta Revisado. As associações foram estimadas por meio da regressão de Poisson.

Resultados

A média do Índice de Qualidade da Dieta Revisado foi de 75,2 pontos (IC95%: 74,2-76,1), com diferença estatisticamente significativa entre os sexos ($p=0,03$). As mulheres obtiveram melhor pontuação para frutas inteiras e sódio ($p<0,01$), enquanto os homens obtiveram maiores escores para óleos, oleaginosas e gordura de peixe ($p=0,02$). Indivíduos com idade igual ou superior a 30 anos obtiveram maior pontuação para o Índice de Qualidade da Dieta Revisado total, fruta inteira, gordura saturada e calorias provenientes de gordura sólida, álcool e açúcar de adição ($p<0,01$). A regressão de Poisson entre o Índice de Qualidade da Dieta Revisado elevado e variáveis independentes mostrou associação para mulheres, para indivíduos com 30 anos ou mais e para aqueles cujo chefe da família apresentava maior escolaridade.

Conclusão

Os fatores que se mostraram associados ao Índice de Qualidade da Dieta Revisado elevado foram a idade, o sexo e a escolaridade do chefe da família.

Termos de indexação: Adulto. Dieta. Consumo alimentar.

INTRODUCTION

In the last decades in Brazil, changes in lifestyle, economic, social, and demographic conditions promoted changes in diet and level of physical activity that affected people's health negatively. These changes are associated with the growing prevalence of obesity and Chronic Non-Communicable Diseases (NCD)^{1,2}.

The need of understanding the association between diet and NCD led to the development

and continuous improvement of dietary indices. In Brazil, Previdelli *et al.*³, used the Brazilian Food Guide⁴ to propose the Brazilian Healthy Eating Index-Revised (BHEI-R), which resulted in the adaptation of the North American Healthy Eating Index (HEI) to the Brazilian Population⁵.

Like the Healthy Eating Index⁵ revised in 2005, the BHEI-R includes the foods and nutrients that are most likely to promote health and prevent disease. The BHEI-R also approaches dietary factors that are considered unhealthy, allowing

their association with socioeconomic, demographic, anthropometric, and lifestyle components; and health-related outcomes.

The Brazilian Healthy Eating Index-Revised³ estimates dietary adequacy and detects changes in dietary patterns; it considers the recommendations for a healthy diet taking into account the Brazilian dietary characteristics and allows the dietary assessment and monitoring of individuals and populations. This index classifies individuals into dietary categories by grouping them into similar segments. The purpose is to investigate possible associations with other factors and to identify population groups that are at risk of consuming a diet inadequate in a given nutrient or food group.

The objective of this study was to analyze the dietary quality of adults living in the urban region of *Cuiabá* (MT), Brazil and identify possible associated factors using the BHEI-R adapted for the Brazilian population.

METHODS

Study design and population

This cross-sectional study included 195 adults of both genders aged 20 to 50 years from the urban area of *Cuiabá* (MT)⁶. A secondary analysis was done of their food intake data, collected by a Food Frequency Questionnaire (FFQ). The sample size was calculated as recommended by Willett *et al.*⁷.

The study participants were selected from a sample of participants of a population-based study that investigated the prevalence of hypertension in the city of *Cuiabá* (MT), in 2003-2004⁸. The present study used cluster sampling in three phases. Stratification was proportional to the density of each urban region of *Cuiabá* (MT). More details about the sampling method can be found elsewhere⁸.

Data were collected from July to September 2007 through personal interviews and

anthropometric assessments done at the participants' homes.

Measurement procedures

The participants' race/skin color was self-reported and classified as recommended by the Instituto Brasileiro de Geografia e Estatística⁹ (IBGE, Brazilian Institute of Geography and Statistics). Age was recorded in years. The individual's and household head's education levels were also classified as recommended by IBGE¹⁰. The socioeconomic level was categorized as recommended by the Associação Brasileira de Empresas de Pesquisa¹¹ (ABEP, Brazilian Association of Survey Companies).

The participants were also classified as active or inactive depending on how much leisure physical activity they had practiced weekly in the month that preceded the interview; other classifications included smoking status (smokers, nonsmokers, or ex-smokers); and consumption of alcoholic beverages based on their consumption in the two weeks that preceded the interview (consumers, non-consumers). More details on their lifestyle-related variables are available elsewhere¹².

Anthropometric and body composition assessments

Weight; height; and waist, and hip circumferences were measured as recommended by the literature^{13,14}. Weight was determined by a body composition analyzer of the brand Tanita, model UM 080, with 150.0kg capacity and 0.1kg accuracy. This same analyzer was used for determining percentage of body fat. Except for weight, anthropometric measurements were taken twice and the final measurement was given by calculating the means. Nutritional status was classified according to Body Mass Index (BMI), given by dividing the weight by the square of the height. BMI, waist circumference, and Waist-to-

Hip Ratio (WHR) were categorized according to the cut-off points recommended by the World Health Organization¹⁵. Percentage of body fat was categorized according to the cut-off points proposed by Gibson¹⁶.

Food consumption

Habitual food intake data were collected by a semiquantitative FFQ. The said FFQ contained 81 food items divided into food groups. Three standard serving sizes and eight intake frequency options, from three times per day to rarely or never, were available for each item. This study investigated 75 food items, leaving out those whose main components had already been reflected by other items.

The intake frequencies reported in the FFQ were converted into daily frequencies and a value of 1.0 was attributed to a once-daily consumption. Proportional values were used for the other frequency options. The mean daily intake of each food item was calculated by multiplying the daily frequency by the amount consumed.

The energy and nutrient contents of the diet were determined by the software Nutwin¹⁷, which uses the United States Department of Agriculture (USDA) food composition table. The food groups were grouped according to the groups in the Brazilian Food Guide⁴. The preparations that contained more than one food group, such as sandwiches, pizzas, stuffed pasta, and other mixtures, were divided into their individual components, which were then classified in the corresponding groups. The nutrients and dietary information of foods that were not found in the program's database were collected from the Nutrition Data System for Research (NDSR)¹⁸ and from the *Tabela Brasileira de Composição de Alimentos* (TACO, Brazilian Food Composition Table)¹⁹.

Diet quality assessment

The Brazilian Healthy Eating Index-Revised³ was used for assessing diet quality. Originally, this

index consisted of points given to twelve components that characterize different dietary aspects: nine components are food groups; two are nutrients (sodium and saturated fat); and one is the energy percentage of the diet coming from Solid Fats, Alcoholic beverages, and Added Sugar (SoFAAS). The score attributed to the food group components included the number of servings recommended by the Brazilian Food Guide⁴ for a 2,000kcal diet.

The item "*Whole Grains*" in the BHEI-R was not used in the present study because the study FFQ does not distinguish between grain types. Therefore, 10 points were attributed to three 1,000kcal grain servings as a criterion for the maximum score in the item "*Total Grains*". Hence, the BHEI-R in this study consists of points stemming from eleven components.

The total Brazilian Healthy Eating Index-Revised score is 100 points. High scores indicate that the diet is close to ideal, while low scores indicate that the diet is far from ideal.

Statistical analysis

The total and component Brazilian Healthy Eating Index-Revised scores were analyzed as continuous variables. Additionally, the total scores were categorized into quartiles. The continuous variables were expressed as means, standard deviation, and 95% Confidence Interval (95%CI) and the categorical variables as proportion (%) and respective 95%CI. The Kolmogorov-Smirnov test was used for determining the distribution symmetry of the continuous variables. The nonparametric variable means were compared by the Mann-Whitney test ($p < 0.05$).

The bivariate analysis used the prevalence ratio and the 95%CI to investigate possible associations between the independent variables (gender, age, participant's education level, household head education level, social class, marital status, skin color, leisure physical activity status, smoking status, alcohol intake status, BMI, percentage of body fat, waist-to-hip ratio, and

waist circumference) and the response variable (high BHEI-R). The variables with p value ≤ 0.20 in the bivariate analysis were included in the Poisson regression. The significance level was set at 5% ($p \leq 0.05$).

Brazilian Healthy Eating Index-Revised scores above the third quartile were considered high. The independent variables were included simultaneously in the Poisson regression model.

Ethical aspects

The research project was approved by the Research Ethics Committee of the University Hospital Júlio Muller under protocol number nº 234/CEP-HUJM/05, on December 3, 2008. All participants signed a Free and Informed Consent form before they entered the study, as recommended by Resolution 196/96²⁰ of the National Health Council.

RESULTS

A total of 195 adults were assessed; of these, 51% were females, 58% were aged 30 years or more, 80% were not Caucasians, 55% were from the social classes A and B, 54% were single, 74% were from household whose head had more than 8 years of formal education, and 52% were overweight or obese (Table 1).

The mean Brazilian Healthy Eating Index-Revised score was 75.2 points (95%CI=74.2-76.1), and the mean scores of women and men differed significantly (76.1 and 74.2; $p=0.03$). Table 2 shows that men obtained higher scores for the food groups oils, nuts, and fish oil (9.4 and 8.9; $p=0.02$), while women obtained higher scores for whole fruits (4.7 and 4.2; $p<0.01$) and sodium (2.9 and 1.9; $p<0.01$).

Table 2 shows that the participants aged 30 or older had higher total BHEI-R scores (76.4 and 73.6; $p<0.01$), and they had higher scores for the components whole fruit (4.7 and 4.2; $p<0.01$), saturated fat (9.0 and 8.5; $p<0.01$); and

Table 1. Characterization of the sociodemographic, lifestyle, weight, and body composition data of a sample of adults from Cuiabá (MT), Brazil, 2007.

Variable	n	%	95%CI
<i>Gender</i>			
Male	95	48.7	41.5- 56.0
Female	100	51.3	44.0- 58.5
<i>Age (years)</i>			
20 to 29	82	42.1	35.0- 49.3
30 to 39	53	27.2	21.7- 34.0
40 to 50	60	30.8	24.4- 37.8
<i>Marital status</i>			
Single	93	47.7	40.5- 54.9
Married	89	45.6	38.5- 52.9
Widowed	2	1.0	0.1- 3.7
Separated	11	5.6	2.8- 9.9
<i>Social class</i>			
A	21	10.8	6.8- 16.0
B	87	44.6	37.5- 51.9
C	74	37.9	31.1- 45.2
D	13	6.7	3.6- 11.1
<i>Skin color</i>			
White	39	20.0	14.6- 26.3
Brown	103	52.8	45.6- 60.0
Black	49	25.1	19.2- 31.8
Asian	4	2.1	0.6- 5.2
<i>Participant's education level</i>			
≥8 years of formal education	172	88.2	82.8- 92.4
<8 years of formal education	23	11.8	7.6- 17.2
<i>Household head education level</i>			
≥8 years of formal education	145	74.4	67.6- 80.3
<8 years of formal education	50	25.6	19.7- 32.4
<i>Leisure physical activity</i>			
Yes	74	37.9	31.1- 45.2
No	121	62.1	54.8- 68.9
<i>Smoking status</i>			
Smoker	25	12.8	8.5- 18.3
Ex-smoker	29	14.9	10.2- 20.7
Non smoker	141	72.3	65.5- 78.5
<i>Alcoholic beverage intake</i>			
Yes	75	38.5	31.6- 45.7
No	120	61.5	54.3- 68.4
<i>Waist-to-hip ratio</i>			
Normal	179	91.8	87.0- 95.2
High	16	8.2	4.8- 13.0
<i>Percentage of body fat</i>			
Normal	102	21.5	17.1- 26.5
High	93	78.5	72.2- 84.8
<i>Body mass index</i>			
Underweight	6	3.1	1.1- 6.6
Normal weight	87	44.6	37.5- 51.9
Overweight	64	32.8	26.3- 39.9
Obese	38	19.5	14.2- 25.8

Note: 95%CI: 95% Confidence Interval.

calories from solid fats, alcoholic beverages and added sugar (17.0 and 15.1; $p < 0.01$).

Table 3 analyzes the association between explanatory variables and the response variable (high BHEI-R) given by the crude Prevalence Ratio (PR) and its respective 95%CI. Women were more likely to have higher BHEI-R scores than men (PR=2.15; 95%CI=1.17-3.95). Likewise, higher scores of BHEI-R were seen in older individuals (PR=2.23; 95%CI=1.18-4.21), married individuals (PR=2.70; 95%CI=1.47-4.96), individuals whose household head had at least 8 years of formal education (PR=2.06; 95%CI=0.99-4.61), and Caucasians (PR=1.61; 95%CI=0.95-2.83).

Poisson regression between high BHEI-R and the independent variables showed that only

the sociodemographic variables were associated with high BHEI-R scores. Women were twice as likely to have a high BHEI-R (Odds Ratio-OR=2.00; 95%CI=1.08-3.69). Other factors associated with high BHEI-R were age ≥ 30 years (OR=2.07; 95%CI=1.09-3.93) and belonging to a household whose head had 8 years or more of formal education (OR=2.21; 95%CI=1.07-4.92), regardless of other factors (Table 4).

DISCUSSION

In the sample of adults evaluated in *Cuiabá* (MT) the participants with better diets were women, individuals aged 30 years or more, and individuals whose household heads had at least

Table 2. Mean and 95% confidence interval of the Brazilian Healthy Eating Index - Revised (BHEI-R) and its components according to gender and age in a sample of adults from *Cuiabá* (MT), Brazil, 2007.

Components BHEI-R	Maximum score	Total BHEI-R scores	Gender		Age	
			Male	Female	Under 30 years	30 years or more
Total BHEI-R	100	75.16 (74.18-76.14)	74.22 (72.80-75.64)	76.05 (74.71-77.4)*	73.57 (72.06-75.14)	76.44 (75.24-77.64)*
Total Fruit	5	4.55 (4.41-4.70)	4.38 (4.12-4.64)	4.71 (4.57-4.81)	4.4 (4.23-4.71)	4.62 (4.44-4.80)
Whole Fruit	5	4.45 (4.27-4.62)	4.18 (3.88-4.49)	4.68 (4.52-4.85)*	4.18 (3.88-4.48)	4.66 (4.47-4.85)*
Total vegetables	5	4.61 (4.49-4.73)	4.67 (4.51-4.83)	4.54 (4.36-4.71)	4.51 (4.31-4.71)	4.69 (4.54-4.83)
Dark green and orange vegetables	5	4.15 (3.95-4.36)	4.29 (4.01-4.57)	4.02 (3.71-4.32)	4.06 (3.73-4.40)	4.23 (3.96-4.50)
Total grains	10	6.43 (6.18-6.68)	6.41 (6.08-6.75)	6.44 (6.07-6.82)	6.44 (6.07-6.80)	6.43 (6.09-6.77)
Meats, eggs, legumes	10	9.94 (9.86-10.00)	9.94 (9.84-10.00)	9.92 (9.82-10.00)	10.00 (10.00-10.00)	9.88 (9.75-10.00)
Milk	10	4.49 (4.11-4.87)	4.23 (3.65-4.81)	4.73 (4.23-5.22)	4.66 (4.09-5.22)	4.36 (3.84-4.87)
Oils	10	9.13 (8.90-9.37)	9.37 (9.05-9.62)*	8.9 (8.55-9.26)	9.2 (8.84-9.60)	9.06 (8.75-9.38)
Saturated fat	10	8.81 (8.63-9.00)	8.81 (8.52-9.10)	8.81 (8.55-9.06)	8.54 (8.23-8.85)	9.03 (8.81-9.26)*
Sodium	10	2.43 (2.08-2.79)	1.87 (1.41-2.33)	2.96 (2.43-3.48)*	2.35 (1.83-2.86)	2.50 (2.00-2.99)
SoFAAS	20	16.16 (15.47-16.85)	16.00 (15.03-16.97)	16.30 (15.30-17.3)	15.14 (14.03-16.25)	16.98 (16.12-17.83)*

Note: * $p < 0.05$ associated according to the Mann-Whitney test.

SoFAAS: Calories from solid Fats, Alcoholic beverages, and Added Sugar.

Table 3. Prevalence of high Brazilian Healthy Eating Index-Revised (BHEI-R) score (%), Prevalence Ratio (PR), and 95% Confidence Interval (95%CI), according to the characteristics of a sample of adults from Cuiabá (MT), Brazil, 2007.

Variable	%	PR (95%CI)	p value
<i>Gender</i>			
Female	34.0 (34/100)	2.15 (1.17 - 3.95)	0.01
Male	15.8 (15/95)	1.00	
<i>Age</i>			
>30 years	33.3 (36/108)	2.23 (1.18 - 4.21)	0.01
≤30 years	14.9 (13/87)	1.00	
<i>Participant's education level</i>			
≥8 years	26.2 (45/172)	1.50 (0.54 - 4.18)	0.43
<8 years	17.4 (4/23)	1.00	
<i>Household head education level</i>			
≥8 years	29.0 (42/145)	2.06 (0.99 - 4.61)	0.08
<8 years	14.0 (7/50)	1.00	
<i>Social class*</i>			
A and B	26.9 (29/108)	1.17 (0.66 - 2.06)	0.59
C and D	23.0 (20/87)	1.00	
<i>Marital status</i>			
Married	38.2 (34/89)	2.70 (1.47 - 4.96)	0.001
Not married	14.2 (15/106)	1.00	
<i>Skin color</i>			
White	33.9 (21/62)	1.61 (0.91 - 2.83)	0.05
Non-white	21.1 (28/133)	1.00	
<i>Leisure physical activity</i>			
Yes	25.7 (19/74)	1.04 (0.58 - 1.84)	0.91
No	24.8 (30/121)	1.00	
<i>Smoking status</i>			
No	26.8 (45/168)	1.74 (0.63 - 4.84)	0.29
Yes	15.4 (4/26)	1.00	
<i>Alcoholic beverage intake</i>			
No	27.0 (17/63)	1.13 (0.63 - 1.99)	0.69
Yes	24.0 (18/75)	1.00	
<i>BMI (kg/m²)¹</i>			
Excess weight	27.5 (28/102)	1.22 (0.69 - 2.14)	0.50
No excess weight	22.6 (21/93)	1.00	
<i>Waist-to-hip ratio¹</i>			
At risk	37.5 (6/16)	1.56 (0.66 - 3.67)	0.31
No risk	24.0 (43/179)	1.00	
<i>Percentage of body fat²</i>			
At risk	30.1 (28/93)	1.46 (0.83 - 2.57)	0.19
No risk	20.6 (21/102)	1.00	
<i>Waist circumference¹</i>			
At risk	29.3 (24/82)	1.37 (0.78 - 2.41)	0.28
No risk	21.4 (24/112)	1.00	

Note: *Economic classification according to the Brazilian Association of Survey Companies (ABEP)¹¹; ¹World Health Organization cut-off points¹⁵; ²Gibson cut-off points¹⁶. BMI: Body Mass Index.

Table 4. Crude (PR_c) and adjusted (PR_{ad}) Prevalence Ratios and 95% Confidence Interval (95%CI) between the Brazilian Healthy Eating Index-Revised (BHEI-R) and associated factors. *Cuiabá* (MT), Brazil, 2007.

Variables	PR _c	95%CI	PR _{ad}	95%CI
<i>Gender</i>				
Female/Male	2.15	1.17 - 3.95	2.00	1.08 - 3.69
<i>Age</i>				
≥30 years/<30 years	2.23	1.18 - 4.21	2.07	1.09 - 3.93
<i>Household head education level (in years)</i>				
≥8/<8	2.06	0.99 - 4.61	2.21	1.07 - 4.92

8 years of formal education. Analysis of the BHEI-R components shows that the consumption of fruits and non-starchy vegetables was close to the recommended intakes, but the consumption of sodium was high, and the consumption of milk and dairy products was low.

There are no other Brazilian studies that use the BHEI-R in adults. However, comparison of studies that use the HEI-2005 and BHEI-R is possible because the two indices are similar.

Results similar to those of the present study were found by other studies done in Brazil and elsewhere, that is, different HEI scores between genders, age groups, and education level of the household head^{21,22}. Gender, age group, and education level are determinants of food habits since they influence an individual's decision to adopt protective or risk behaviors concordant with the cultural standards of his group. People become more health aware as they age, which may have a positive influence on diet quality, contributing to better food choices. Regarding education level, the observed association may be explained by the relationship between low education level and low income, and by lower access to nutrition information^{23,24}.

The use of a Food Frequency Questionnaire for collecting dietary data may have affected the scores of some BHEI-R components because the main limitations of this method are its predefined food list and its dependence on the participants' understanding and memory²⁵. However, the FFQ reflects the usual intake, which minimizes

intraindividual variability, contrary to the 24-hour Recall (R24h) or the food record.

The mean Brazilian Healthy Eating Index-Revised scores found by the present study, 74.2 for men and 76.1 for women, was greater than those found for North American adults, which varied from 52.6 to 65.4 points^{26,27}. In a study conducted by Pires²⁸ with 204 individuals from both genders and aged 18 to 79 years participating in a population-based study in the municipality of *São Paulo*, the mean BHEI-R was 64.0 points for men and 65.4 points for women. The mean BHEI-R scores found by the present study may have been overestimated because of the method used for collecting dietary data. According to the literature, the mean HEI score tends to be overestimated when the FFQ is used, contrary to the R 24h²⁹.

In the present study, the mean score of the group meats, eggs, and legumes was close to 10 points for both genders. Similar results were seen in adults from *São Paulo* (SP)²⁸. The consumption of meats in Brazil has been increasing according to the food acquisition per household data published by the *Pesquisa de Orçamento Familiar* (POF, Family Budget Surveys)³⁰ that show that between 2002/2003 and 2008/2009, the percentage of household expenditure on meats, including organ meats and fish, increased from 18.3% to 21.9% of the total food expenditures. The trend of high meat, eggs and legumes consumption has also been observed abroad²⁶. Another factor that may justify the high scores for this group is the fact that HEI-2005

and BHEI-R add legumes to the groups of meats and eggs, resulting in an overestimation of protein intake.

The intake of whole fruits, total fruits, total vegetables, dark green and orange vegetables and legumes was high, contrary to literature data²⁶⁻²⁸. It is possible that the use of a FFQ to collect dietary data contributed to the overestimated consumption of these items. Data from POF 2008/2009³⁰ show that less than 10.0% of the Brazilian population reaches the recommended intakes for fruits and non-starchy vegetables. Data from Vigitel³¹ show that the frequency of regular consumption of fruit and non-starchy vegetables was 29.9% of the adult population, being lower in men (24.7%) than in women (34.4). The study also shows that the consumption of fruits and non-starchy vegetables of both genders increase with age and education level.

The estimated scores for the group milk and dairy products in this study (4.49) were comparable to the results obtained by a study in another country³². In Brazil, Morimoto *et al.*²¹ found a score of 2.9 for the group milk and dairy products. The Inquérito Nacional de Alimentação (INA, National Food Survey) done in 2008/2009³⁰ evidenced that the consumption of milk and dairy among Brazilian adults is much lower than ideal, which means a high prevalence of inadequate vitamin and calcium intakes.

Determining sodium intake is challenging because it is hard to measure the amount of sodium added to foods, so the reported intake is inaccurate. Sodium intake was high in this study; both genders had a low score, especially males. These scores are lower than the mean scores found by other studies done in Brazil and abroad^{26,28,33}. According to the POF 2008-2009³⁰ data, 88.7% of men and 69.7% of women aged 19 to 59 years consumed too much sodium daily (>2300mg/day), and most of the sodium came from processed foods.

Many studies associate high sodium intake with the development of chronic diseases and indicate that if individuals aged 25 to 55 years

reduced their daily sodium intake by 1300mg, their systolic blood pressure would drop by 5mmHg and the prevalence of high blood pressure would drop by 20.0%. Low sodium intake could also reduce stroke (14.0%) and coronary artery (9.0%) mortalities worldwide³⁴. The population-based sample that gave rise to this study sample had a high prevalence of high blood pressure (28.3%), and the prevalence was higher in males (33.5%) than in females (23.5%)⁸.

One of the limitations of the present study is its cross-sectional design because it is not possible to infer relationships of causality. However, it is possible to explore the factors associated with diet quality. Another limitation is the possible low FFQ accuracy, not only because its number of food items is limited, but also because it usually overestimates food intake, which is also influenced by recall bias.

On the other hand, a positive aspect of the study was the way the sample was selected, since the selection was based on a population-based study, which contributed to its internal validity. This study is original in this region, and its results show that the diet quality of the participants is not ideal, especially with respect to sodium intake. Therefore, monitoring and surveillance are necessary, given the high prevalence of high blood pressure in the study municipality.

CONCLUSION

The mean Brazilian Healthy Eating Index-Revised scores found by the present study were higher than those found by other Brazilian and foreign studies. Sodium presented the most inappropriate intake pattern. Better diet quality was directly associated with higher age, being female, and belonging to a household whose head had a higher education level. Possible result errors can be minimized by future studies by using methods other than the FFQ for collecting food intake data, especially methods that do not rely on the memory of the participants.

CONTRIBUTORS

AS LOUREIRO performed the study, statistical analysis, and data interpretation; and wrote the manuscript. RMVG SILVA analyzed and interpreted the results. PRM RODRIGUES analyzed and interpreted the results. RA PEREIRA analyzed and interpreted the results; and wrote the manuscript. LL WENDPAP analyzed and interpreted the results. MG FERREIRA designed and supervised the study, performed the statistical analysis, interpreted the data, and wrote the manuscript.

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Transgenic and conventional Brazilian soybeans don't cause or prevent preneoplastic colon lesions or oxidative stress in a 90-day *in vivo* study

Sojas transgênicas e convencionais brasileiras não causam ou previnem lesões pré-neoplásicas ou stress oxidativo em estudo in vivo de 90 dias

Felipe Augusto SBRUZZI¹

Vinícius de Paula VENÂNCIO²

Maria Cristina Costa RESCK²

Maísa Ribeiro Pereira Lima BRIGAGÃO³

Luciana AZEVEDO⁴

ABSTRACT

Objective

The study presents the results of a 90-day safety assessment of rats fed with four varieties of soybeans, BRS 245 RR and BRS *Valiosa* RR (transgenic), BRS 133 and MG BR46 *Conquista* (non-transgenic).

Methods

Diets were prepared by incorporating toasted soybean flour to a commercial diet at 1%, 10% or 20% weight. In the *in vivo* experimental the rats' body weight, body weight gain, food consumption, number of aberrant crypt foci, oxidative stress biomarkers, urea and creatinine levels were analyzed and compared between experimental groups, as well as histopathological observations (digestive tract, liver, kidneys).

¹ Universidade Federal de Alfenas, Faculdade de Ciências Farmacêuticas. Alfenas, MG, Brasil.

² Universidade José do Rosário Vellano, Departamento de Medicina Veterinária. Alfenas, MG, Brasil.

³ Universidade Federal de Alfenas, Instituto de Ciências Exatas, Programa de Pós-Graduação em Ciências Cirúrgicas. Alfenas, MG, Brasil.

⁴ Universidade Federal de Alfenas, Faculdade de Nutrição, Departamento de Nutrição. R. Gabriel Monteiro da Silva, 700, 37130-000, Alfenas, MG, Brasil. *Correspondência para/Correspondence to:* L AZEVEDO. *E-mail:* <luciana.azevedo@unifal-mg.edu.br>.

Results

The results indicate that glyphosate-tolerant soy varieties neither induce nor prevent aberrant crypt foci induction, nor do their conventional counterparts. Similarly, none of the four soybean varieties tested induced changes in the digestive tract, liver or kidney. Serum biochemical parameters were also unchanged.

Conclusion

The consumption of both, conventional and transgenic soybeans, were insufficient to ameliorate dimethylhydrazine-induced oxidative stress.

Indexing terms: Aberrant crypt foci. Colon carcinogenesis. Dimethylhydrazine. Free radicals. Genetically Modified Organisms. Transgenic soybean.

RESUMO

Objetivo

Este estudo apresenta os resultados de um experimento de 90 dias com o objetivo de avaliar a segurança de quatro variedades de grãos de soja: BRS 245 RR e BRS Valiosa RR (transgênicas), BRS 133 e MG BR46 Conquista (não transgênicas).

Métodos

As dietas foram preparadas incorporando farinha de grãos de soja à dieta comercial (FRI-LAB Ratos II) a 1%, 10% ou 20% m/m. O peso corpóreo dos animais, o ganho de peso, o consumo de dieta, o número de focos de criptas aberrantes e os níveis de marcadores de estresse oxidativo, de creatinina e de ureia foram comparados entre os grupos experimentais, assim como as observações histopatológicas (trato digestivo, fígado e rins).

Resultados

Os resultados indicaram que as variantes glifosato-tolerantes não induziram ou preveniram a indução de focos de criptas aberrantes, assim como suas parentais convencionais. Similarmente, nenhuma das quatro variedades de grãos de soja testadas induziu alterações no trato digestivo, no fígado e nos rins. Os parâmetros bioquímicos do soro permaneceram também inalterados.

Conclusão

Tanto o consumo de grãos de soja convencionais quanto o de transgênicos foram ineficazes para melhorar os níveis de estresse oxidativo induzidos pela dimetilhidrazina.

Termos de indexação: *Focos de criptas aberrantes. Carcinogênese de cólon. Dimetilhidrazina. Radicais livres. Organismo geneticamente modificados. Soja transgênica.*

INTRODUCTION

According to the International Service for the Acquisition of Agri-Biotech Applications, during 2011, an additional 12 million hectares of biotech crops were cultivated, representing an annual growth rate of 8% percent over 2010. Among them, the soybean stands out as the major biotech crop as Genetically Modified Organisms (GMO), occupying 75% of global adoption rates. In this setting, Brazil is the second largest world producer of soy, behind only the United States¹. The increasing supply of transgenic soybeans and the great versatility of its use in many foods

determine the growing consumption by the population. Despite the potential benefits of transgenic techniques, serious concerns have been raised related to the potential environmental and medical consequences of GMO use. The medical consequences, or risk factors, are generally assigned to health risks of conventional foods, including toxicity, allergenicity, anti-nutrition effects, unintended side-effects, the secondary effects of gene expression, and the disruption of the genetic material or metabolism of the consumer². The risk of mutagenesis and modified gene expression or even cancer itself, in the long term, is the biggest fear of most populations.

Safety assessment of genetically modified crops initially focuses on comparisons with the nearest isogenic relative using agronomic performance metric and compositional analysis to determine whether the particular genetic modification produces unintended effects³. Therefore, nutritional and safety assessments of GMO foods are one of the key measures that nutritionists can take to allay the fears of the public.

To assess carcinogenesis risk, parameters such as Aberrant Crypt Foci (ACF) can be evaluated. In numerous animal studies, ACF predicts subsequent development of colorectal cancer⁴. Furthermore, oxidative stress parameters are associated with cancer development and progression⁵. Colorectal tissue is regularly exposed to a variety of hazardous chemicals, and the Reactive Oxygen/Nitrogen Species (ROS/RNS) formed during the metabolic transformation of these compounds has been thought to play an important role in carcinogenesis⁶. Then, this study was designed to compare different contents of transgenic and conventional soybeans as potential colon lesion-inducing or protective agents. It was also explored whether the consumption of soybeans could ameliorate previously established colon lesions and associated oxidative damages.

METHODS

Chemical reagents

1,2-Dimethylhydrazine (DMH), 1,1,3,3-Tetraethoxypropane (TEP), reduced GSH, Glutathione Desulfide (GSSG), glutathione reductase (E.C.1.6.4.2), β -Nicotinamide Adenine Dinucleotide Phosphate, reduced (NADPH), Monobromobimane (mBrB), guanidine and Butylhydroxytoluene (BHT) were obtained from Sigma Aldrich Chemical Co. Methanol and acetic acid were purchased from J.T. Baker, Inc. All other reagents were analytical grade.

Soybeans cultivars

The following Brazilian soybeans were used: BRS 245 RR (Transgenic Soybean 1) and its most closely related non-transgenic strain, BRS133 (Conventional Soybean 1); also, MGBRS Valiosa RR (Transgenic Soybean 2) and its most closely related non-transgenic strain, MGBR46 Conquista (Conventional Soybean 2) were also evaluated.

Although all the soybeans were certified, a Roundup Ready test was conducted to confirm the presence or absence of the glyphosate-resistant CP4-5-enolpyruvyls-hikimate-3-phosphate-synthase (CP4 EPSPS) protein with the AgraStrip[®] GMO RR test kit from Romer Labs[®].

Diet preparation

The diets used were prepared according to the following procedure: the soybeans (8% weight/weight - w/w moisture) were dehydrated in a cabinet dryer at 100°C, 30 minutes. The dehydrated grains were ground in a mill with 0.5mm mesh at approximately 3,000xg. The flour was then transferred back to the cabinet dryer for another 30 min (4% w/w moisture). After these procedures, the dehydrated soy flour was mixed with a commercial diet at 1%, 10% or 20% (w/w)⁷.

Compositional analysis of all the diets used was performed in triplicate. After the diet preparation, moisture was determined by loss while drying with an Infrared IV200 Moisture Analyzer for 8 minutes at 120°C. Total nitrogen was analyzed by the Kjeldahl procedure (conversion factor was 6.25) and ash content was determined by incineration at 550°C in a muffle furnace. Total fat was estimated according to Bligh & Dyer⁸ and the carbohydrates were calculated as the remainder (difference using the fresh weight-derived)⁹.

Experimental Protocol and determination of ACF number

The animals used in this study were handled in accordance with the Ethical Principles

for Animal Research adopted by the *Colégio Brasileiro de Experimentação Animal* (COBEA, Brazilian College of Animal Experimentation). The protocol used herein was approved by the *Universidade Federal de Alfenas, Minas Gerais* Ethics Committee for Animal Research (94/2006). Two experiments were performed, being the first one designed to assess the activity of transgenic and conventional soybean on the modulation of preneoplastic lesion in colon (induction effect study). The second experiment was conducted to determine whether transgenic and conventional soy could be used to reduce the damage in the colon (chemopreventive test). For this purpose, rats were randomly distributed into 14 groups of 8 animals each for the induction effect study (Figure 1A) and the chemopreventive test (Figure 1B), during 13 weeks. The inducing drug, DMH, was dissolved in 0.9% weight/volume(w/v) NaCl immediately before use. The 0.9% (w/v) NaCl and DMH were given in 2 doses of 40mgkg⁻¹ b.w. for week, during 2 weeks. For both experiment, groups 1 (negative control groups) received commercial diet for the entire experimental period and groups 2 (positive control groups) received commercial diet and DMH. The experimental groups received diets containing different

soybeans types and concentrations throughout the experimental period.

For Aberrant Crypt Foci analysis, the colons were obtained, stained with methylene blue and analyzed according to Fenoglio-Preiser & Noffsinger⁴. Fifty sequential fields on 40×magnification of the distal colon were screened for ACF, which were distinguished by an elongated, slit-shaped lumen surrounded by thickened epithelium that stained more intensively than the surrounding normal crypts. The number of ACF and the number of Aberrant Crypts (AC) in the distal colon were recorded.

Biochemical parameters

For the oxidative stress determination, the serum containing 0.05% BHT was quickly frozen until MDA (Malonaldehyde) analysis. Samples were chromatographed after standard preparation according to Brown & Kelly¹⁰. Fluorimetric detection was performed (λ_{ex} 532nm, λ_{em} 553nm) and peak of Malondialdehyde-Thiobarbituric Acid (MDA-TBA) adduct was calibrated with 97% (v/v) TEP.

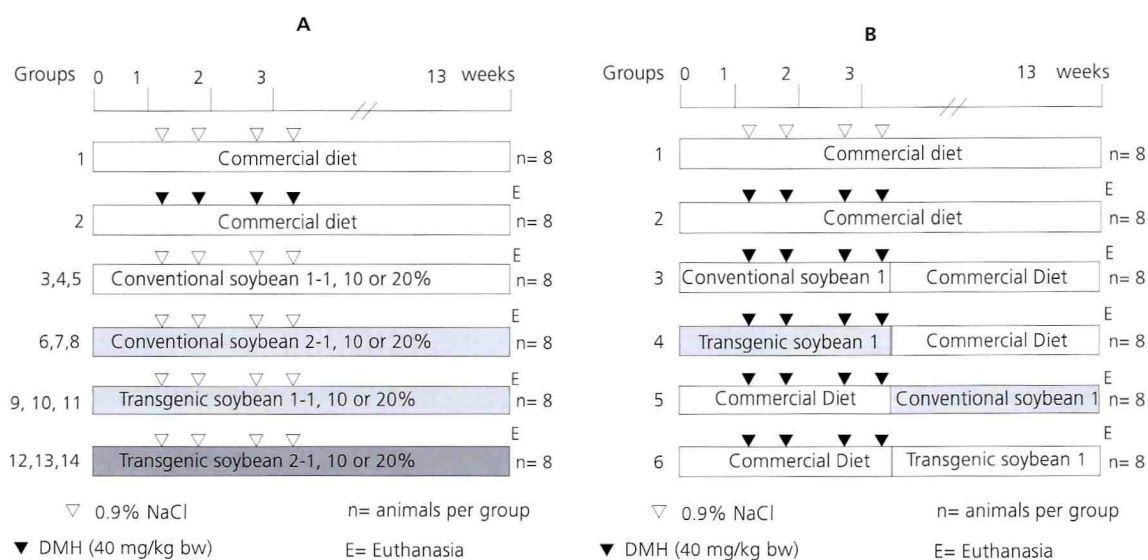


Figure 1. Protocols for (A) the inductive effect experiment of soybeans and (B) the chemopreventive experiment of soybeans in colon carcinogenesis.

Protein Carbonyl (PC) was measured by the formation of protein hydrazone derivatives from the reaction with Dinitrophenylhydrazine (DNPH), which were quantified spectrophotometrically. Separate blanks were prepared without DNPH and the carbonyl¹¹ content was determined from the equation $\epsilon_{370\text{nm}} = 22,000 \text{ M}^{-1}\text{cm}^{-1}$.

GSH and other thiols react with mBrB, a sulfhydryl-alkylating agent. To investigate possible changes in the level of GSH resulting from soy ingestion and DMH treatment, thiol adducts with mBrB were separated by High-Performance Liquid chromatography (HPLC)¹². GSH was identified by comparison to the retention times of authentic standards as measured by fluorescence detector ($\lambda_{\text{ex}} 360\text{nm}$, $\lambda_{\text{em}} 470\text{nm}$) and its quantification was performed through integral analysis of areas. GSSG content was determined through treatment with GSSG reductase coupled with NADPH. Its stoichiometry with GSH was calculated and expressed as the ratio GSH (GSH+0.5 GSSG)⁻¹. All parameters were standardized for total protein content¹³.

The creatinine and urea levels were determined in all samples by routine spectrophotometric methods with compound-specific kits (BioTecnica®).

Histopathological analysis

Sections of each lobe of the liver, small intestine and stomach were taken immediately after animal euthanasia. The tissues were fixed in buffered 4% (v/v) formaldehyde for 24 hours, dehydrated in graded ethanol and embedded in paraffin. Thin sections (5 μm) were obtained, stained with hematoxylin-eosin and examined microscopically for histological changes.

Statistical analysis

The body weight, weight gain, food consumption measured weekly and centesimal composition were analyzed by Analysis of

Variance (Anova). The total number of ACF, number of AC per focus and the relationship between crypt/FCA in the different groups were analyzed by the Kruskal-Wallis test. Two-way Anova was used to compare serum oxidative stress parameters between groups.

For statistical analyses, *p* values equal to or lower than 0.05 were considered statistically significant.

RESULTS

The transgenic grains, flours and diets were positive for the CP4 EPSPS protein and the conventional products were negative for this protein. This analysis confirmed the certified origin of the grains and, importantly, that the conventional soybeans were GMO free. The centesimal composition of the experimental diets demonstrated that the diets with the same percentages of soybeans were similar to each other regardless of whether they contained transgenic or conventional soybeans, as shown in Table 1.

In both experiments, differences in body weight, weight gain and food consumption were observed during the DMH injection period, but by the end of the experiment, the induced groups did not show any differences from other groups.

The body weight gain ranged between 290 \pm 31g and 333 \pm 19g in the induction effect study, and between 259 \pm 28g and 298 \pm 30g in the chemopreventive test. The consumption of all soybean cultivars, transgenic or not, was very similar in both experiments. On this way, the average daily consumption of soybean in the groups that received diet at 1%, 10% or 20% (w/w) soybeans was 1 \pm 0g/kg⁻¹ b.w., 9 \pm 3g/kg⁻¹ b.w. and 18 \pm 6g/kg⁻¹ b.w., respectively.

In the induction effect study only the positive group showed ACF, counting 6 \pm 3 for the total number of ACF, and 2 \pm 0 for the total number of AC and Crypt/ACF. The numbers of ACF and crypt multiplicity in the colon from

Table 1. Compositional equivalence of the studied diets in g/kg⁻¹*

Diet	Soybeans (%)	Fat		Protein		Ash		Moisture		Carbohydrate	
		M	SD	M	SD	M	SD	M	SD	M	SD
Commercial Diet	—	40.3	2.5	229.0	0.8	78.4	0.8	111.7	3.1	541.0	4.0
Conventional soybean 1	1	40.2	0.2	233.9	1.4	84.8	0.5	114.7	2.5	527.0	3.0
	10	50.9	0.7	250.6	2.6	78.0	2.7	127.7	2.9	493.0	5.0
	20	66.0	1.7	268.4	2.6	72.9	0.3	124.7	3.8	468.0	5.0
Transgenic soybean 1	1	39.8	1.0	234.4	1.3	83.8	2.5	128.7	2.1	513.0	4.0
	10	53.0	2.6	256.7	0.5	75.9	2.1	124.3	1.5	490.0	4.0
	20	62.4	3.0	273.1	2.1	73.2	0.4	125.7	2.1	466.0	4.0
Conventional soybean 2	1	41.0	1.0	235.1	1.2	82.9	0.3	116.7	4.7	524.0	5.0
	10	52.9	3.1	257.9	1.2	78.8	2.4	126.7	2.3	485.0	5.0
	20	63.1	2.5	273.1	2.0	73.5	0.4	118.0	3.5	472.0	5.0
Transgenic soybean 2	1	39.4	1.3	234.0	4.2	84.8	1.6	119.3	2.1	522.0	4.0
	10	54.7	3.4	254.9	2.7	76.9	0.3	121.0	5.2	522.0	4.0
	20	65.4	2.6	268.5	2.2	70.8	1.9	109.3	3.2	486.0	5.0

Note: *Values are given as Mean (M) ± Standard Deviation (SD). No statistical difference among diets with the same soybean content.

Table 2. Chemopreventive test: Lack of anticarcinogenicity of a soybean-containing diet on aberrant crypt foci formation and crypt multiplicity (crypt/ACF) in colon^a.

Group/Treatment ^b	Total n° ACF		Total n° AC		Crypt/ ACF	
	M	SD	M	SD	M	SD
Commercial diet + NaCl	1	2 [†]	1	4 [†]	0	1 [†]
Commercial diet + DMH	16	8	34	17	2	1
Conventional soybean 1 + DMH	10	5	27	14	3	1
Transgenic soybean 1 + DMH	10	8	25	18	2	1
DMH + Conventional soybean 1	11	7	26	19	2	1
DMH + Transgenic soybean 1	13	3	29	8	2	1

Note: ^aValues are given as mean (M) ± Standard Deviation (SD). ^b0.9% NaCl (0.1 mL 10 g⁻¹ b.w.); DMH: 1,2-dimethylhydrazine (4 × 40 mg/kg⁻¹ b.w.). [†]p < 0.05 (Kruskal-Wallis): the negative control group was statistically different from the other groups; AC: Aberrant Crypts; ACF: Aberrant Crypt Foci.

different groups of chemopreventive experiment are summarized in Table 2. The results show that all DMH-initiated animals developed ACF by the 13th week of the experiment.

Lipid peroxidation and protein oxidation biomarkers revealed that DMH is an oxidative stress inductor (Tables 3 and 4). Typical chromatograms obtained from the MDA-TBA adduct showed an average retention time of 5.53 min. The basal lipid peroxidation level detected in serum samples was 12.9 ± 0.8 nmol MDA mg⁻¹ protein, while DMH-treated animals showed roughly doubled concentrations of MDA. Also, oxidative damage to protein structure is evident in DMH-treated animals; PC levels of

DMH-treated animals increased nearly twice as much as those of control group. However, the ratio of GSH (GSH+0.5 GSSG)⁻¹ detected in serum samples as GSH-mBrB adduct (retention time 12.96 minutes) was not altered by DMH treatment. The mean value of this ratio was 0.25 ± 0.02, clearly indicating that no GSH oxidation or consequent GSSG accumulation occurred in serum samples under these experimental conditions.

From the same data, it is clear that soybeans ingestion was unable to induce or prevent oxidative damage in lipid and serum structures, or to modify redox serum status.

Table 3. Effect of soybean consumption on oxidative stress parameters¹.

Group/treatment ^b	Soybeans (%)	nmol MDA mg protein ⁻¹		nmol PC mg protein ⁻¹		Ratio GSH (GSH + 0.5 GSSG) ⁻¹	
		M	SD	M	SD	M	SD
Commercial diet + DMH	—	24.8	3.9 [*]	19.2	2.1 [*]	0.199	0.128
Commercial diet	—	12.9	0.8	8.7	1.6	0.289	0.028
Conventional soybean 1	1	14.2	0.7	8.4	0.9	0.309	0.018
	10	16.4	0.5	7.7	2.4	0.254	0.108
	20	11.9	0.4	7.3	71.1	0.314	0.020
Conventional soybean 2	1	12.1	0.8	6.9	2.6	0.239	0.021
	10	13.5	0.7	8.4	1.1	0.319	0.108
	20	15.6	0.3	8.7	1.6	0.244	0.088
Transgenic soybean 1	1	17.9	0.8	9.3	1.0	0.310	0.029
	10	12.9	0.7	8.2	0.6	0.235	0.021
	20	15.9	0.5	9.7	1.9	0.307	0.037
Transgenic soybean 2	1	12.9	0.7	9.3	2.6	0.294	0.480
	10	16.9	0.8	8.9	1.0	0.314	0.020
	20	11.9	0.9	9.6	1.4	0.239	0.021

Note: ^{*} $p < 0.05$ (Two-way Anova): The DMH-treated group (1,2-dimethylhydrazine; $4 \times 40 \text{ mg/kg}^{-1}$ b.w.) was statistically different from the other groups (treated with 0.9% NaCl ($0.1 \text{ mL } 10 \text{ g}^{-1}$ b.w.)). ^aValues are given as Mean (M) \pm Standard Deviation (SD); DMH= 1,2 – Dimethylhydrazine ($4 \times 40 \text{ mg/kg}^{-1}$ b.w.)

MDA: Malondialdehyde; PC: Protein Carbonyl; GSH: Glutathione; GSSG: Glutathione Disulfide.

Table 4. The effect of combined DMH treatment and soybeans diet on serum oxidative stress biomarkers^a.

Group/treatment ^b	nmol MDA mg protein ⁻¹		nmol PC mg protein ⁻¹		Ratio GSH (GSH + 0.5 GSSG) ⁻¹	
	M	SD	M	SD	M	SD
Commercial diet + NaCl	12.9	0.8 [*]	8.7	1.6 [*]	0.289	0.028
Commercial diet + DMH	24.8	3.9	19.2	2.1	0.199	0.128
Conventional soybean 1 + DMH	22.9	4.2	18.3	4.1	0.209	0.068
Transgenic soybean 1 + DMH	27.9	3.4	18.7	3.6	0.259	0.880
DMH + Conventional soybean 1	25.9	1.9	19.3	5.1	0.214	0.020
DMH + Transgenic soybean 1	31.1	2.7	17.7	4.6	0.189	0.171

Note: ^{*} $p < 0.05$ (Two-way Anova): The negative control group was statistically different from the other groups.

^aValues are given as mean \pm SD; ^b0.9% NaCl (w/w) ($0.1 \text{ mL } 10 \text{ g}^{-1}$ b.w.); DMH = 1,2-dimethylhydrazine ($4 \times 40 \text{ mg.kg}^{-1}$ b.w.).

Neither creatinine nor urea serum levels were altered by DMH treatment or the soybeans diet. Creatinine, a normal product from metabolic creatine breakdown, was detected at a mean value of $0.46 \pm 0.018 \text{ mg/dL}^{-1}$, and serum urea was detected at $41.56 \pm 12.8 \text{ mg/dL}^{-1}$. Both biochemical markers were good indicators that renal function was unaltered in the animals.

Liver, kidney, small intestine, pancreas and stomach histopathological analysis indicated that none of the soybeans-based diets were toxic to

these organs. No congestion, edema, cellular reactions (lymphocytic infiltration), cell degeneration or necrosis were observed in any of the analyzed tissues (data not shown).

DISCUSSION

Kok *et al.*¹⁴ state that new protein and other types of expression products or metabolites that have been introduced into new plant varieties and are not already part of the human diet should be

assessed for their safety to the human consumer. The essence of this approach is that the new food (or component thereof) should be compared with appropriate conventional strain that is already accepted as safe based on its history as food¹⁵.

However, in spite of the inquiries associated with GMO, soy could be classified as a putative chemopreventive due to the allegations about its functional properties associated with health-promoting phytochemicals, which have antioxidant activities. According to Zeiger¹⁶ on the other hand, many substances reported to be antimutagens or anticarcinogens have themselves been shown to be mutagenic, carcinogenic or oxidant in many systems. Considering these factors, the present study was performed to investigate the safety and potential anticarcinogenic properties of transgenic soybean flours, in comparison to their nearest conventional counterparts. We investigated its ability to either induce or prevent ACF, histological lesions and serum oxidative stress parameters. Additional priority has to be given to measuring biomarkers of general oxidative changes correlated with the initiation and progression of the targeted cancer.

Despite the substantial equivalence principle, unintended adverse effects in edible fractions of Genetically Modified (GM) crops may occur even if they were not identified in composition or agronomic studies¹⁷. To address this possibility, animal feeding studies have been conducted with foods obtained from GM crops to determine whether they result in unintended deleterious nutritional changes. Meanwhile, the results of our study show that no biologically meaningful differences occur in the *in vivo* nutritional response variables of body weight, body weight gain and food consumption when comparing the testing groups to negative controls and when comparing groups with the same concentration of soybeans in the diet. These response variables serve as a sensitive indicator of general animal well being³. These results suggest that, under our experimental conditions,

the four varieties of soy (two conventional and two transgenic) were nutritionally equivalent, providing evidence that the transgenic strains did not have cause unintended deleterious nutritional changes in the animals. This nutritional equivalence also confirms studies that substituting 11% and 33% of the diet with soy will not cause significant nutritional changes in rodents¹⁸.

Our results further showed that neither transgenic nor conventional soybeans diets stimulated or suppressed the development of ACF and crypt multiplicity at any of the levels tested. In the chemopreventive test (Table 2), we also evaluated whether transgenic and conventional soy can modify carcinogenesis before and after induction with carcinogen treatment. This was based on the knowledge of cancer development: cancer preventative agents can be classified based on their ability to affect preneoplastic lesions of different stages either before or after the carcinogen treatment. Furthermore, beneficial effects for health have been found for soy isoflavones, proteins and fibers^{19,20}. Conversely, Omar *et al.*²¹ also observed that soybean flour enhanced carcinogenesis and promoted the progression of established neoplasms; in a second study²², an increase in tumors were reported in DMH-treated rats fed genistein.

Histopathological analyses of the digestive tract and the ACF observations revealed no evidence of lesions in soy-fed rats compared to the negative control group. Identical results were observed in the liver and kidneys, which are the main biotransformation and excretion organs. These data reinforce the idea that diets containing conventional or transgenic soybeans were not able to promote or prevent tissue injury.

Among biochemical pathways that mediate cancer, oxidative injury has been suggested as a relevant mechanism for damage that might contribute to neoplasia, a common final consequence related to this pathology. As MDA is one of the major secondary oxidation products derived from polyunsaturated fatty acids, its measurement has been regarded as a reflection

of the lipid peroxidation level. This oxidative process generates a complex variety of products, many of them reactive electrophiles. These products can react with protein and DNA, causing toxicity and mutagenicity²³. As shown in Table 3, DMH induced a significant increase in MDA serum levels (about 2-fold), presenting strong evidence that this carcinogenic substance causes lipid damage in parallel with the occurrence of preneoplastic lesions. A study in humans conducted by Spadafranca *et al.*²⁴ demonstrates that after 42 days of soy diet (80mg day⁻¹ of isoflavones) no significant effect on MDA level was observed. Other reports have found antioxidant effects attributable to soy isoflavones or other compounds present in this grain²⁵. In our experiment, neither the conventional soybeans nor the transgenic ones had any impact on MDA levels (Table 4).

Protein oxidation results from ROS/RNS attack on protein structures, leading to a variety of structural and functional damage, which can be measured through DNPH adduction. Consistent with oxidative stress induced by DMH, about 2.2 times more PC was detected in serum of animals submitted to DMH treatment than in control animals (Table 3). Soybean ingestion was incapable of decreasing or preventing a rise in this serum biomarker (Table 4).

GSH primarily functions as a non-enzymatic reducing agent, preventing oxidative stress in most cells and body fluids, and helping to trap ROS/RNS that can damage lipids, proteins, carbohydrates and nucleic acids. There is a direct correlation between the ratio of reduced glutathione/total glutathione and the redox state of the organism because GSH is converted to GSSG when an oxidative environment predominates over the antioxidant capability of the cells⁵. In concordance with the two other oxidant biomarkers, MDA and PCO, the GSH parameter was the same in animals fed with conventional or transgenic soybeans and no statistical difference is observed when compared to control groups (Table 3 and 4).

The lack of anticarcinogenic effect of soy in the tested situations may be explained by several conditions. First of all, the primary location of action of any orally administrated compound is the digestive tract. Later, the effects of absorption and distribution and the impacts on individual organs can be observed, and, finally, the kinetics of metabolism and excretion may have effects on the body^{26,27}. The effects of chemopreventive dietary constituents are under the direct influence of the food matrix and their technological processing²⁸, once these factors may affect their interaction with intestinal biotransformation enzymes²⁶. It is possible that bioactive soybean compounds could not exert their chemopreventive effects due to absorption in the small intestine, which would not allow a substantial amount to reach the lumen of the colon and prevent preneoplastic lesions *in loco*. It is also possible that the ingested compounds were not bioavailable or that they did not have any effect on colon carcinogenesis. It is also important to consider that the intestinal microbiota is variable in different animal conditions, and different microorganisms may have diverse abilities to generate bioactive compounds from isoflavones and other compounds found in foods²⁹.

CONCLUSION

Taken together, our results support the conclusion that neither BRS 245 RR nor BRS Valiosa RR transgenic soybeans stimulated or suppressed the development of ACF and crypt multiplicity, as well as non-transgenic grains, and show that soy antioxidant phytochemicals are unable to protect against dimethylhydrazine-induced lipid and protein oxidative damage.

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CONTRIBUTORS

FA SBRUZZI contributed in the data obtaining, analysis and interpretation and in the manuscript writing. VP VENANCIO contributed in the data analysis and interpretation and also in the manuscript writing. MCC RESCK contributed in the execution and interpretation of histopathological analysis. MPRL BRIGAGÃO contributed in the execution and interpretation of oxidative stress analysis, in addition to the manuscript writing. L AZEVEDO contributed to the study design, data analysis and manuscript writing.

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Factors associated with iron deficiency in pregnant women seen at a public prenatal care service

Fatores associados à deficiência de ferro em gestantes atendidas em serviço público de pré-natal

Rosângela Maria Souza de CAMARGO¹

Rosângela Alves PEREIRA²

Edna Massae YOKOO³

Janine SCHIRMER⁴

ABSTRACT

Objective

This study aimed to determine the frequency of iron deficiency and its association with socioeconomic, obstetric, and nutritional factors in pregnant women.

Methods

This cross-sectional study included women on the second trimester of pregnancy seen at a public prenatal care facility of *Cuiabá, Mato Grosso*, Brazil from May 2008 to May 2009. Socioeconomic, nutritional, and obstetric data were compared with markers of iron stores.

Results

During the study period, 146 pregnant women met the inclusion criteria. The frequency of anemia characterized by abnormal hemoglobin level, hematocrit, and mean corpuscular volume varied from 3% to 5%. However, 11% of the women had high transferrin levels and 39% had low ferritin levels. Before pregnancy, 21% of the women were underweight and 29% were overweight or obese. During pregnancy, the percentage of overweight or obese women rose to 40%. History of miscarriages, higher gestational age, and excess weight before pregnancy were associated with markers of iron stores at abnormal levels. Consumption of specific food groups was not associated with abnormal marker levels.

¹ Universidade Federal do Mato Grosso, Faculdade de Nutrição, Departamento de Alimentos Nutrição. Av. Fernando Correa da Costa, 2367, Boa Esperança, Campus Universitário, 78060-900, Cuiabá, MG, Brasil. *Correspondência para/Correspondence to:* RMS CAMARGO. *E-mail:* <rosanms@terra.com.br>.

² Universidade Federal do Rio de Janeiro, Instituto de Nutrição Josué de Castro. Rio de Janeiro, RJ, Brasil.

³ Universidade Federal Fluminense, Instituto de Saúde da Comunidade. Niterói, RJ, Brasil.

⁴ Universidade Federal de São Paulo, Escola Paulista de Enfermagem. São Paulo, SP, Brasil.

Conclusion

Serum ferritin was the most sensitive indicator of iron deficiency. Excess weight and anemia were concomitant.

Indexing terms: Anemia. Ferritins. Food intake. Iron deficiency. Nutritional status. Pregnancy.

RESUMO

Objetivo

Verificar a frequência de deficiência de ferro e sua associação com fatores socioeconômicos, obstétricos e nutricionais em gestantes.

Métodos

Estudo transversal desenvolvido com gestantes no segundo trimestre da gestação atendidas em ambulatório de pré-natal da rede pública de Cuiabá, Mato Grosso, entre maio de 2008 e maio de 2009. Dados sobre as características socioeconômicas, nutricionais e obstétricas foram relacionados aos indicadores de reservas de ferro.

Resultados

No período do estudo, 146 gestantes atenderam aos critérios de inclusão na pesquisa. A frequência de anemia variou de 3% a 5%, considerando-se os valores para hemoglobina, hematócrito e volume corpuscular médio. Entretanto, a frequência de alterações nos indicadores de reservas de ferro variou de 11% para transferrina a 39% para ferritina. No período pré-gestacional, 21% das gestantes apresentavam baixo peso e 29% excesso de peso (sobrepeso ou obesidade); no período gestacional, a ocorrência de excesso de peso aumentou (40%). Histórico de aborto, idade gestacional e status de peso pré-gestacional se associaram às alterações nos indicadores de reservas de ferro. Não se observou associação entre o consumo de grupos de alimentos e alterações nos indicadores de reservas de ferro.

Conclusão

O indicador mais sensível na identificação da deficiência de ferro foi a ferritina sérica. Observou-se concomitância de excesso de peso e deficiência de ferro.

Termos de indexação: Anemia. Ferritina. Consumo Alimentar. Deficiência de Ferro. Estado Nutricional. Gravidez.

INTRODUCTION

According to the World Health Organization¹, half of all pregnant women develop anemia, which is characterized by a hemoglobin level lower than 11g/dL. The mean prevalence of anemia in pregnant women from developed and developing countries is 18% and 56%², respectively, but it may be as high as 75% in India³.

In Brazil, anemia is the most common nutritional problem, especially in children under two years of age and in pregnant women, with prevalences of 50% and 35%, respectively². The National Survey on the Demography and Health of Children and Women reported that 30% of the women of childbearing age are anemic and that there are no national studies with consistent

data about the occurrence of anemia in pregnant women⁴. In a review study, Côrtes *et al.*⁵ reported that the prevalence of anemia in Brazilian women on the second trimester of pregnancy varies from 9% to 44%.

The primary causes of anemia during pregnancy are inadequate intake of dietary iron⁶, greater fetal demand, and increased blood volume during pregnancy⁷. Although iron absorption is high during pregnancy, the amount of dietary iron absorbed and mobilization of the iron stores are not enough to meet the demand⁸. However, the etiology of anemia during pregnancy in developing countries also includes factors such as low socioeconomic and education levels⁴, and high parity⁶.

Iron deficiency is associated with high morbidity and mortality rates. Furthermore,

anemia impairs mental development and the ability to work and study productively⁹. During pregnancy, anemia is associated with a higher rate of mother/fetus morbidity and mortality, and the most common complications are early labor, low birth weight, preeclampsia, and higher risk of miscarriage¹⁰.

The objective of this study was to investigate whether socioeconomic, obstetric and nutritional factors are associated with anemia/iron deficiency in pregnant women.

METHODS

This cross-sectional study was done at the outpatient clinic of the *Hospital Universitário Júlio Muller* (HUJM) of the *Universidade Federal do Mato Grosso* (UFMT), *Cuiabá, Mato Grosso, Brazil*, which is a reference hospital for low- and high-risk pregnancies.

The study was approved by the Research Ethics Committee (*Comitê de Ética e Pesquisa - CEP*) of the *Universidade Federal de São Paulo* (Unifesp) under protocol number 1468/CEP/Unifesp/2007 and of HUJM under protocol number 384/CEP/HUJM/2007. All participants signed a free and informed consent form before they entered the study.

Study design and population

The study population consisted of pregnant women seen at the HUJM prenatal care outpatient clinic. Women aged 19 to 49 years on the second trimester of their first pregnancy seen between May 2008 and May 2009 were eligible. Women with the following pre-pregnancy conditions were excluded: kidney, liver, heart, pulmonary, and endocrine diseases; infectious diseases, such as tuberculosis and Acquired Immunodeficiency Syndrome (AIDS); high blood pressure; diabetes *Mellitus*; and obstetric diseases, such as preeclampsia, placental abruption, and bleeding. The gestational ages were collected

from ultrasound reports in the participants' medical records. The second trimester of pregnancy was defined as the period from week 14 to week 28 of gestation.

Women in the first trimester of pregnancy were excluded because gastrointestinal symptoms, such as nausea, vomiting, dyspepsia, and heartburn, usually encourage them to change their food habits¹¹.

A pretested questionnaire was used to collect demographic, socioeconomic, reproductive, and dietary data during individual interviews with the participants. The interviewers were trained dieticians submitted to regular refresher training sessions during the data collection period. Some data were collected from the medical records.

The dependent variables were serum Hemoglobin level (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), and the following markers of iron stores: Serum Iron (Fe), Serum Ferritin (SF), Serum Transferrin (TRF), Total Iron-Binding Capacity (TIBC), and Transferrin Saturation (TSAT). The iron status of the participants was categorized according to the number of the said markers outside of the recommended ranges: women with an abnormal level of one marker and women with an abnormal level of two or more markers.

The independent variables were age, education level, marital status, per capita household income, gestational age, first prenatal care visit, number of prenatal care visits, parity, abortions, pre-pregnancy weight status, pregnancy weight status, and food intake.

Data collection

Blood was collected from the participants after a 10-hour fast to determine the levels of anemia and iron store markers. The biochemical tests were performed by the HUJM laboratory. The methods and equipment used for the tests, the cut-off point for each marker, and the classification of the blood markers are listed in Chart 1.

Chart 1. Reference values and classification of the blood components.

Blood components	Analysis method	Cut-off points	Nutritional status classification
Hemoglobin	Pentra 80 spectrophotometer, ABX, (Paris, France).	Below 11g/dL	Anemia
Hematocrit	Numeric integration of the mean corpuscular volume.	Below 33%	Anemia
Mean Corpuscular Volume	Calculated directly from the red blood cell count.	Below 80fl	Anemia
Serum iron	Automated colorimetric method (BT 3000, Wiener Lab Group, Argentina).	Below 67mg/dL	Iron deficiency
Serum ferritin	Chemiluminescence (Modular E170 - ROCHE, Frankfurt, Germany).	Below 22mg/dL	Iron deficiency
Serum transferrin	Nephelometry (concentration of an emulsion is given by comparing its transparency with that of a standard preparation) (BT 3000, Wiener Lab Group, Argentina).	Above 400mg/dL	Iron deficiency
Total iron-binding capacity	Automated colorimetric method (BT 3000, Wiener Lab Group, Argentina).	Above 300mg/dL	Iron deficiency
Transferrin saturation	Given by the formula: TSAT (%)=serum iron/TIBCX100.	Below 16%	Iron deficiency

Note: TSAT: Transferrin Saturation; TIBCX: Total Iron Binding Capacity.

Weight status before and during pregnancy was classified according to the Body Mass Index (BMI) cut-off points. BMI is given by dividing weight by the square of the height. Weight status before pregnancy was classified according to the Institute of Medicine (IOM)¹² cut-off points, and weight status during pregnancy was classified according to the cut-offs proposed by the Brazilian Ministry of Health¹³.

A validated Food Frequency Questionnaire (FFQ) developed for the adult population of another city located in the Brazilian Central-West Region¹⁴ was used for collecting the participants' food intake data because no FFQ validated for the city of *Cuiabá* (MT) was available at the time. The study FFQ was adapted by excluding some food items and including some dietary iron sources and typical local foods. The original FFQ contained 80 items, and after the adaptation there were 72 items. The FFQ was administered qualitatively, providing seven options for intake frequency: (a) Once daily; (b) two or more times daily; (c) five to six times weekly; (d) Two to four times weekly; (e) once weekly; (f) one to three times monthly; and (g) rarely or never.

For the analysis, the foods were grouped according to their nutritional characteristics, iron bioavailability, and ability to promote iron absorption: iron sources (liver, gizzard, chicken heart, corned beef, beef, and beans), absorption facilitators (orange, acerola berry, lime and cashew apple), and absorption inhibitors (milk, dairy products, soda, tea, and coffee). Therefore, 19 food groups were defined: milk and dairy products, meats and eggs, oils and fats, snacks and canned foods, grains, rice pasta, legumes, non-starchy vegetables, cooked vegetables, tubers and roots, fruits, sugary foods, natural and artificially-flavored juices, tea and coffee, soda, organ meats, iron sources, iron absorption facilitators, iron absorption inhibitors. The reported food intake frequencies were converted into daily frequencies and analyzed as continuous variables. For this purpose, a value of 1 was attributed to foods eaten once daily and the other frequencies were given proportional values, as follows: twice or more daily (2); five to six times weekly $[(5+6)/2]/7=0.79$ times per day; two to four times weekly $[(2+4)/2]/7=0.43$ times per day; once weekly $(1/7)=0.14$ times per day; one to three times per month $[(1+3)/2]/30=0.07$ times daily; and rarely or never (0).

Statistical analyses

The continuous variables were expressed as means, standard deviations, and 95% confidence intervals. Proportions were calculated for the categorical variables. The relative anemia frequency was estimated. The frequencies of pregnant women with only one marker of iron stores and those with two or more markers of iron stores at abnormal levels were also calculated.

The normality of the distributions was assessed by the Kolmogorov-Smirnov test, and the means with symmetrical and nonparametric distributions were compared by the Student's *t* test and Mann-Whitney test, respectively. The categorical variables were compared by the Chi-square test or Fisher's exact test when necessary. The significance level was set at 5%.

RESULTS

A total of 221 pregnant women were eligible for the study. Of these, 36 (16%) missed the interview and 39 (18%) did not undergo the biochemical tests. Hence, the final sample consisted of 146 pregnant women, that is, 66% of the initial sample.

The mean red blood counts and serum iron markers were within the normal levels. The frequency of anemia characterized by abnormal MCV, Hb, or Hct levels varied from 3% to 5%.

However, the anemia frequencies characterized by abnormal levels of one or more iron markers were higher, varying from 11% for transferrin to 39% for ferritin (Table 1). Only one pregnant woman did not have any abnormal levels of anemia or anemia markers; 38% (*n*=55) of the women had one marker at abnormal level, and 62% (*n*=90) had two or more markers at abnormal levels (data not shown).

Iron deficiency was not associated with age, education level, income, marital status, parity, and occasion of the first prenatal care visit. Participants who had had miscarriages were more likely to have low serum iron levels (*p*=0.03) and low TSAT (*p*=0.02). Women pregnant for more than 20 weeks were more likely to have low ferritin (*p*=0.01) and high transferrin (*p*=0.006) levels. Low ferritin level was associated with the number of prenatal care visits: 52% of the women who had attended at least two prenatal care visits and 24% of those who attended only one visit had low ferritin levels (*p*=0.01). Finally, women who were overweight or obese before getting pregnant were also more likely to have low serum iron levels (*p*=0.04) (Table 2).

Pregnant women who were overweight or obese (76%) before getting pregnant were more likely to have two or more markers of anemia at abnormal levels than those who were underweight (65%) or normal weight (53%) (*p*=0.04). There was no association between

Table 1. Markers of serum iron and iron stores in pregnant women seen in public prenatal care facilities of Cuiabá (MT), Brazil, 2009.

Marker	n*	M	SD	95%CI	Anemia frequency
Hemoglobin (g/dL)	146	12.1	0.74	12.0; 12.2	5
Hematocrit (%)	146	36.1	2.27	35.8; 36.5	4
Mean corpuscular volume (fl.)	146	89.2	4.89	88.4; 89.9	3
Iron (µg/L)	143	86.0	31.5	80.7; 91.2	30
Serum ferritin (µg/L)	141	39.2	36.1	33.2; 45.2	39
Transferrin (µg/dL)	133	329.3	61.4	318.7; 39.8	11
Transferrin saturation (%)	142	29.7	14.0	27.3; 32.0	16
Total iron-binding capacity (µg/dL)	142	273.3	77.5	260.5; 286.2	30

Note: M: Mean; SD: Standard Deviation; 95%CI: 95% Confidence Interval.

*The sample size for each marker varied because some biochemical tests were lost.

Table 2. Association between socioeconomic and obstetric characteristics and abnormal levels of markers of iron stores in pregnant women (n=146) seen at a public prenatal care facility of *Cuiabá* (MT), Brazil, 2009.

Participant characteristics	%	Abnormal levels of markers of iron stores ¹ (%)				
		Serum ferritin	Serum iron	Transferrin	TSAT	TIBC
<i>Age (years)</i>						
<25	47	21	12	11	7	13
≥25	53	19	19	13	10	17
<i>p</i> value ²		0.25	0.15	0.75	0.60	0.71
<i>Education level (years)</i>						
≤8	36	41	26	11	14	30
>8	64	40	34	13	19	31
<i>p</i> value ²		0.89	0.30	0.71	0.45	0.86
<i>Per capita household income (minimum salaries)</i>						
≤1	60	40	31	12	22	36
>1	40	41	3	13	9	22
<i>p</i> value ²		0.90	0.93	0.84	0.05	0.08
<i>Marital status</i>						
No partner	19	46	23	4	11	30
Has partner	81	39	33	14	18	30
<i>p</i> value ²		0.51	0.34	0.17	0.37	0.93
<i>Parity</i>						
None	41	37	29	14	14	32
One or more	59	43	32	10	19	29
<i>p</i> value ²		0.52	0.67	0.50	0.37	0.67
<i>Miscarriages</i>						
Yes	37	45	42	14	26	38
No	63	38	24	11	11	26
<i>p</i> value ²		0.36	0.03	0.58	0.02	0.13
<i>Gestational age (weeks)</i>						
<20	57	21	27	3	15	32
≥20	43	56	34	19	18	29
<i>p</i> value ²		0.01	0.37	0.006	0.61	0.76
<i>First prenatal care visit (weeks)</i>						
≤12	58	46	29	17	16	30
>12	42	32	34	6	19	31
<i>p</i> value ²		0.08	0.50	0.06	0.64	0.96
<i>Prenatal care visits</i>						
1	42	24	28	7	17	25
≥2	58	52	33	15	17	34
<i>p</i> value ²		0.01	0.49	0.17	0.99	0.28
<i>Pre-pregnancy weight status</i>						
Underweight	21	52	29	10	10	29
Normal weight	50	34	23	13	15	26
Excess weight	29	44	45	11	26	38
<i>p</i> value ²		0.22	0.04	0.88	0.13	0.40
<i>Gestational weight status</i>						
Underweight	21	52	20	7	7	32
Normal weight	40	35	31	15	16	21
Excess weight	40	40	36	11	22	38
<i>p</i> value ²		0.34	0.30	0.59	0.20	0.16

Note: ¹Only one participant had no marker of anemia or iron stores outside the recommended range. ²Chi-square test.

TSAT: Transferrin Saturation, TIBC: Total Iron-Binding Capacity.

Table 3. Weight status and abnormal levels of markers of anemia and iron stores in pregnant women (n=146) seen at a public prenatal care facility of *Cuiabá* (MT), Brazil, 2009.

Weight status	Total (n=146) %	Number of markers of anemia/iron stores ¹ at abnormal levels		
		1 (n=55) %	≥2 (n=90) %	p value ²
<i>Before pregnancy</i>				
Underweight	21	35	65	0.04
Normal weight	50	47	53	
Excess weight*	29	24	76	
<i>During pregnancy</i>				
Underweight	20	30	70	0.28
Normal weight	40	46	54	
Excess weight	40	35	66	

Note: *Partitioned Chi-square test ($p=0.013$). ¹Only one participant had no marker of anemia or iron stores outside the recommended range; ²Chi-square test.

Table 4. Daily food group intake frequency according to the number of markers of iron stores at abnormal levels in pregnant women (n=145)* seen at a public prenatal care facility of *Cuiabá* (MT), Brazil, 2009.

Food	One marker of anemia/iron stores outside the recommended level (n=55)		≥2 markers of anemia/iron stores outside the recommended level (n=90)	
	Food group daily intake frequency			
	M	95%CI	M	95%CI
Rice and pasta	2.0	1.9-2.2	2.0	1.8-2.1
Legumes (beans)	2.4	2.0-2.8	2.5	2.1-2.9
Grain-based products (bread, cookies, cakes)	1.7	1.5-1.9	1.9	1.7-2.2
Tubers and roots	0.6	0.4-0.8	0.5	0.3-0.6
Milk and dairy products	1.5	1.2-1.7	1.4	1.2-1.6
Meats and eggs	2.1	1.9-2.4	2.0	1.8-2.2
Organ meats	0.1	0.1-0.2	0.2	0.1-0.2
Non-starchy vegetables	1.3	1.1-1.5	1.2	1.0-1.4
Cooked vegetables	0.7	0.3-1.1	0.5	0.4-0.7
Fruits	0.7	0.5-0.9	0.8	0.6-0.9
Sugary foods	0.8	0.6-1.1	0.7	0.6-0.9
Natural and artificially-flavored juices	0.9	0.7-1.1	1.0	0.9-1.2
Milk and coffee	0.9	0.7-1.2	0.8	0.7-0.10
Soda	0.5	0.3-0.6	0.5	0.4-0.7
Oils and fats	2.1	1.9-2.3	2.1	2.0-2.3
Snacks and canned foods	1.1	0.9-1.3	1.1	0.9-1.3
Iron sources	2.4	2.2-2.6	2.2	1.1-2.4
Iron-absorption inhibitors	3.2	2.8-3.6	3.0	2.7-3.3
Iron-absorption facilitators	1.1	0.8-1.3	1.3	1.1-1.5

Note: M: Mean; 95%CI: 95% Confidence Interval.

*Only one participant had no marker of anemia or iron stores outside the recommended range.

gestational weight status and level of iron stores (Table 3).

Specific food habits were not associated with markers of anemia/ iron stores at abnormal levels (Table 4).

DISCUSSION

Relatively low anemia frequencies were observed in pregnant women on the second trimester of pregnancy seen at a public prenatal

care service. However, more specific tests showed high frequencies of iron stores. The analyses showed that ferritin was the most sensitive marker of iron stores. The consumption of specific food groups was not associated with low iron stores. Nonetheless, two or more markers of anemia or iron deficiency at abnormal levels were more common in women who were overweight or obese before pregnancy. A history of miscarriages, gestational age, and excess weight before pregnancy were associated with iron stores.

Anemia is detected during pregnancy by measuring MCV, Hb and Hct. Serum hemoglobin level is the main parameter routinely used by public prenatal care services for diagnosing anemia¹⁵. However, the iron stores of pregnant women decrease before their hemoglobin levels do^{16,17}. The study results show that anemia may not be detected during prenatal care and that other more effective means of diagnosing iron deficiency anemia should be included in the prenatal care routine.

The main markers of iron stores are ferritin, serum iron level, transferrin, TIBC and TSAT. Serum ferritin level is considered the method of choice for assessing anemia and monitoring its treatment^{1,18} because of its high sensitivity and relative affordability¹⁹. On the other hand, serum iron level has small clinical value because of its high variability throughout the day in normal individuals. Transferrin is a plasma protein responsible for iron transport. It holds 0.1% of the total body iron and also presents important variations during the day¹⁸. TIBC and TSAT tests complement serum iron test. TSAT corresponds to the amount of iron available for erythropoiesis. When iron stores are low, TSAT level is low²⁰ and TIBC level is generally high¹⁶.

The diagnosis of iron status during pregnancy is also impaired because there is no consensus in the literature regarding which markers best characterize iron deficiency anemia, which is even more critical during pregnancy because of the blood dilution process¹⁹. These

factors make it difficult to compare studies on the prevalence of anemia in pregnant women.

The study results are comparable to those of Karaoglu *et al.*¹⁷ who assessed 823 pregnant women in Turkey and found that those with serum Hb<11g/dL also had lower serum iron, ferritin and transferrin levels. Additionally, the present findings are similar to those of Dani *et al.*⁹ regarding the frequency of anemia characterized by low serum ferritin level, which was of 38.2% in 102 pregnant women receiving prenatal care in public facilities of *Rio Grande do Sul*, Brazil .

Bressani *et al.*¹⁹, on the other hand, found that 45.9% of 318 pregnant women seen at an outpatient prenatal care clinic of *Recife* (PE), had anemia, but not anemia characterized by low serum ferritin levels. Serum ferritin level may increase during acute and chronic inflammatory diseases, alcohol abuse, and cancer, conditions which are commonly seen in regions where infectious and parasitic diseases are endemic¹⁶. These factors may have contributed to the differences found between the results of the present study and those of Bressani *et al.*¹⁹.

Although the present study tried to control the effect of gestational age on the biochemical markers by assessing only women on the second trimester of pregnancy (14 to 28 weeks), these markers changed over time, that is, abnormal ferritin or transferrin levels were more common in women pregnant for more than 20 weeks. These results corroborate Cruz²¹, who found that ferritin levels decrease as gestational age increases.

A study with 772 pregnant women seen at primary health care facilities of the district of *Butantã* in the city of *São Paulo* also found a higher prevalence of iron deficiency in women with higher gestational ages, characterized by a decrease in the mean Hb levels from one trimester of pregnancy to the next²². In the said study, the authors emphasized that the main cause for lower hemoglobin levels was blood dilution and pointed out that other markers need to be assessed for

differential anemia diagnosis, especially MCV and red blood Cell Distribution Width (RDW). RDW is a marker of anisocytosis, an index of Red blood cell size variation²². This marker was not assessed by the present study, which may be considered a study limitation given its importance for the assessment of iron status.

Padilha *et al.*²³ did not find an association between pre-pregnancy body mass index and anemia during pregnancy. However, in agreement with the present study, the aforementioned authors also observed that the frequency of anemia in women with gestational overweight or obesity increased gradually over time.

The mean daily intake of dietary iron sources by the participants was low. Vasconcelos *et al.*²⁴ showed that pasta, bread, cookies, and cakes were the most common iron-fortified foods consumed by pregnant women seen at the Hospital da Universidade de Brasília, and the frequencies of overweight and obesity among these women were also high. Although wheat flour is considered a good iron vehicle, consumption of fortified foods should be prescribed with caution given the high frequencies of excess weight in pregnant women and the general population. New studies are needed to identify other iron and folic acid vehicles with lower energy densities.

Another limitation of this study is that ferrous sulfate supplementation was not assessed. The study prenatal care service routinely prescribes ferrous sulfate to all pregnant women but the supplement may not be taken correctly. Other data would be necessary to assess the role of ferrous sulfate on the markers of iron status.

The study results allow the identification of pregnant women at greater risk of having low iron stores, even when anemia markers are within the normal ranges. These results are also relevant for the development of routine dietary and nutritional counseling strategies for pregnant women seen at public prenatal care facilities to prevent and treat anemia.

CONCLUSION

The frequency of anemia characterized by the hemoglobin levels of pregnant women on the second trimester of pregnancy seen at a public prenatal care facility of Cuiabá (MT), was of 5%. However, the frequency of iron deficiency characterized by low serum ferritin level was of 39%. Pregnant women with a history of miscarriages, those at 20 or more weeks of pregnancy, and those who were overweight or obese before pregnancy were more likely to have iron deficiency. Consumption of specific food groups was not associated with anemia.

CONTRIBUTORS

RMS CAMARGO conceived the study, collected, treated, analyzed, and interpreted the data, and conceived, wrote, and reviewed the manuscript. RA PEREIRA: analyzed and interpreted the data; conceived the manuscript; and reviewed the manuscript. EM YOKOO analyzed and interpreted the data; and reviewed the manuscript. J SCHIRMER: conceived the study; analyzed and interpreted the data; and reviewed the manuscript.

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Olhares sobre a alimentação contemporânea: a gastro-anomia e os corpos de Botero

Looking at contemporary food: Gastro-anomy and Botero's bodies

Michelle MEDEIROS¹
Alex GALENO¹

RESUMO

O excesso de discursos e informação em torno do tema da alimentação gera um cenário em que o comensal já não sabe como decidir pelo que comer. O sociólogo francês Claude Fischler, partindo do conceito durkheimiano, criou o neologismo gastro-anomia para falar sobre o paradoxo que vivenciamos frente à alimentação contemporânea: nunca soubemos tanto sobre os efeitos da alimentação sobre o corpo e, curiosamente, nunca tivemos tantos problemas de saúde relacionados a ela. É sobre este fenômeno gastro-anômico que trata o presente ensaio. Como a anomia se apresenta na sociedade contemporânea quando o assunto é alimentação? Como nutricionistas entendem esta questão? A arte, por realizar a síntese das propriedades intrínsecas da sociedade, foi utilizada como fio condutor das reflexões. O ponto de partida foi análise de duas obras de épocas distintas: Idade Média e contemporaneidade. O casal Arnolfini representado por Jan van Eyck é uma obra que data do século XV, época em que o discurso médico e o religioso eram aqueles que majoritariamente regiam as escolhas alimentares dos indivíduos. A paródia de Botero, por sua vez, traz à tona corpos volumosos, como uma referência ao excesso: o excesso de informações com que se depara o comensal hoje.

Termos de indexação: Alimentação. Arte. Comportamento alimentar.

ABSTRACT

Excess discourses and information on food results in commensals not knowing what to eat. The Durkheim concept led the French sociologist Claude Fischler to create the neologism gastro-anomy to refer to the paradox that we face today regarding food: we have never known so much about the effects of food on the body and, curiously, we have never had so many food-related health problems. The present essay is about this gastro-anomy phenomenon. How does anomy present itself in today's society with respect to food? How do dieticians understand this issue? Since art synthesizes the intrinsic properties of a society, these reflections

¹ Universidade Federal do Rio Grande do Norte, Centro de Ciências Humanas, Letras e Artes, Programa de Pós-Graduação em Ciências Sociais. *Campus das Ciências da Saúde*, Av. Gal. Gustavo Cordeiro de Farias, s/n., Petrópolis, 59012-570, Natal, RN, Brasil. Correspondência para/Correspondence to: M MEDEIROS. E-mail: <medeiros.michelle@hotmail.com>.

were guided by art. We started off by analyzing two works from very different time periods: the Middle Ages and today. The Arnolfini couple represented by Jan van Eyck is a work from the 15th century, time when the medical and religious discourse mostly determined people's food choices. On the other hand, Botero's parody elicits plump bodies in reference to excess: the excess information available to commensals nowadays.

Indexing terms: Food. Art. Feeding behavior.

A gastro-anomia: diagnóstico do corpo doente

A situação da modernidade nos apresenta um paradoxo: nunca soubemos tanto sobre os efeitos da alimentação sobre o corpo e, curiosamente, nunca tivemos tantos problemas de saúde relacionados a ela. Excesso de peso e obesidade são bons exemplos. Dados da World Health Organization¹ mostram que, em 2008, 1,5 bilhões de adultos apresentavam sobrepeso. Destes, mais de 200 milhões de homens e quase 300 milhões de mulheres estavam obesos. No Brasil, a Pesquisa de Orçamentos Familiares (POF), de 2008 e 2009, mostra que o quadro não é mais a fuga da normalidade: "excesso de peso foi diagnosticado em cerca de metade dos homens e das mulheres"² (p.23).

É verdade que vivemos em uma época na qual nunca tivemos tanta informação sobre alimentação. Os dados acima, todavia, nos fazem pensar que o excesso de informação parece não nos ajudar a comer melhor. Antes, poderia gerar uma cacofonia de discursos, não raro dissonantes: dietéticos, identitários, publicitários, éticos... A modernidade alimentar criou uma situação em que o campo de decisão alimentar do comensal se apresenta amplo. A nós é concedida autonomia para escolher e a questão do dia passa a ser outra: "o que escolher?". Não sabemos.

Diria Claude Fischler que a autonomia crescente é portadora de anomia³. No estado de anomia, devido a uma divisão do trabalho que não é solidária, as formas de agir não encontram espaço para serem replicadas e tornarem-se hábitos e, em seguida, regras de conduta. As forças reguladoras externas ao indivíduo já não conseguem sustentar a regra. Há uma infinidade delas sob o mesmo tema sendo enunciadas

ao mesmo tempo por órgãos que não estão em contato. O que se diz passa a ser vago e geral. Sem regras, "já não se sabe o que é possível e o que não o é, o que é justo e o que é injusto, quais são as reivindicações e as esperanças legítimas, quais são as que ultrapassam as medidas"⁴ (pp.321).

Já não se sabe, ademais, o que se deve e o que não se deve comer. As opiniões fragmentadas já não formam um todo solidário⁵. Para este estado de ausência de regras concernentes à alimentação, Claude Fischler recorreu ao conceito durkheimiano de anomia e criou o neologismo *gastro-anomia*.

O excesso de fragmentação dos grupos sociais e de individualismos modernos tem contribuído para uma crise civilizatória sem precedentes. Cada vez mais a sociedade se assemelha a um ajuntamento de interesses particulares e individuais, comprometendo a dinâmica de cooperação e coesão social. Para Durkheim⁶, a sociedade necessita de um "cimento social" que religue as partes separadas e configure uma totalidade orgânica. Ao analisarmos a sociedade atual, podemos notar que vivemos um paradoxo. Por um lado, reproduzimos valores e comportamentos coletivos, sobretudo, a partir das imagens midiáticas e padrões de consumo. O corpo a ser visto e consumido simbolicamente age coercivamente sobre aquele que está fora dos padrões. Estar acima do peso, portanto, é não se adequar ao padrão moral imagético da sociedade contemporânea.

Por outro lado, a exigência do padrão do culto ao corpo belo e magro recai sobre a responsabilidade do indivíduo. O corpo que deve emagrecer e a responsabilidade de tornar-se belo apresentam-se como deveres morais individuais e não responsabilidade coletiva. É evidente que a anomia também se verifica quando presenciamos

a obesidade enquanto pandemia na sociedade e suas consequências para a saúde dos indivíduos. Corpos doentes pela fadiga depressiva da melancolia diária, heranças cultural e genética das famílias e as consequentes doenças crônicas não-transmissíveis, são exemplos de doenças contemporâneas que atestam tal anomia. O que não significa que corpos demasiadamente magros ou anoréxicos também não se constituam em corpos que fogem ao padrão coletivo. Enfim, os dois polos expressam sintomas de uma cultura anômica.

Em algumas de suas obras - como, por exemplo, *El (H)omnívoro* e *Comer*, esta última com edição em português -, e em entrevista concedida à antropóloga Mirian Goldenberg, Fischler discorre mais sobre essa categoria, e a apresenta - sem desconsiderar outros condicionantes que incidem sobre o tema -, como uma das vias de se analisar "as dificuldades que as pessoas têm para lidar com a complexidade que se tornaram as práticas e representações alimentares na sociedade contemporânea"⁷ (p.237).

A gastro-anomia é o diagnóstico do corpo doente. Gera um conflito ansioso capaz de afetar o corpo. De anoréxicos a obesos, a desconfortante liberdade anômica gera padrões alimentares que retratam a crise da alimentação na contemporaneidade. É sobre este fenômeno gastro-anômico que trata este ensaio. Como a anomia se retrata na sociedade contemporânea? Como conviver com ela?

Por que a arte?

Entendendo a arte como imagem daquilo que somos e construímos como humanos, como disse certa vez Lévi-Strauss⁸, tomaremos como ponto da partida a comparação de duas obras de arte: a clássica pintura do artista plástico flamengo Jan van Eyck, *Os esposais dos Arnolfini*, pintado em 1434, e sua paródia, o quadro do colombiano

Fernando Botero², *Matrimonio Arnolfini segun van Eyck*, 1997 (Figura 1). As obras, em verdade, serão pretextos para acessar o tema da gastro-anomia. A reflexão que instiga este trabalho é a seguinte: o que a ciência da Nutrição poderia fazer para ajudar os comensais a conviver neste ambiente gastro-anômico?

Não desconsideremos que na arte há um pensamento profundo sobre a condição humana⁹. A arte fala sobre nós. Por isso, ela pode servir-nos como meio de instrução da realidade. As obras de Botero em suas figuras volumosas oferecem-nos a possibilidade de pensar uma



Figura 1. Jan van Eyck, *Os esposais dos Arnolfini*, 1434.

Fonte: <<http://www.nationalgallery.org.uk>>.

² A imagem de Fernando Botero não foi adicionada ao ensaio, pois, encontra-se protegida por direitos autorais, segundo a Lei nº 9.610 de 1998.

sociedade do excesso, com corpos que retratam sua época. Seguindo a proposta de Lévi-Strauss de “modelos reduzidos” poderíamos dizer, portanto, que a obra em questão realiza a síntese das propriedades intrínsecas da sociedade, espacialmente e temporalmente¹⁰. Opera-se pela metáfora: por meio da parte procede-se à compreensão do todo.

O excesso como anomia

O casal Arnolfini representado por Jan van Eyck é uma obra que data da Idade Média, representa provavelmente o momento do casamento do mercador italiano Giovanni Arnolfini e sua noiva Jeanne de Chenany¹¹. A obra, de 1434, marca no mundo das técnicas pictóricas o emprego da pintura a óleo, idealizada por van Eyck. O uso do óleo ao invés do ovo na preparação de tintas, como antes se utilizava, apresentava ao artista a possibilidade de trabalhar mais devagar, logo, com mais exatidão e detalhes. Não à toa Van Eyck atingiu seu triunfo na pintura de retratos: telas pintadas com minúcia e precisão retratando o mundo fora delas, como parece ser o caso da representação dos Arnolfini e sua residência.

Durante este período grande parte dos conhecimentos médicos na Europa partia da Igreja, que tratava o corpo como algo não relevante: tudo deveria ser feito em prol da alma e da vida depois da morte¹². As prerrogativas em relação ao jejum e os alertas quanto ao pecado da gula criavam nos fiéis uma postura de temperança ou, quando não, levava-os a aderir a uma dieta tão restritiva que poderia levá-los à morte, como de fato ocorreu. Conforme Bruno Laurioux, historiador medievalista da alimentação, a religião ocupava o primeiro lugar dentre as normas culturais que incidiam sobre o discurso alimentar na Idade Média¹³.

O artista fornece-nos pistas da religiosidade do casal. Tome-se como exemplo o espelho retratado no fundo da tela. Os medalhões que o contornam representam cenas bíblicas. Além do nome da obra, há sinais nela que indicam que o

tema é uma cerimônia religiosa. O cão pode ser símbolo de fidelidade¹⁴; as laranjas como símbolo de pureza original, como também pensa Carlos Fuentes em *El naranjo*; o lustre tem apenas uma vela acesa, o que pode indicar provavelmente a espiritualidade do ato (Além disso, era costume em Bruges, cidade belga onde vivia o casal, manter acesa uma vela na noite do casamento); os personagens de mãos dadas no centro da pintura denotam que o tema principal da obra era a união do casal¹⁵, o que é reforçado pela maneira como o casal dispõe de suas mãos: “a jovem acaba de pôr sua mão direita sobre a esquerda de Arnolfini, e este parece estar prestes a colocar sua mão direita na esquerda dela, como símbolo solene de sua união”¹¹ (p.240). O reflexo no espelho parece nos indicar que Giovanni recebia pessoas que chegavam para a cerimônia. Os sinais nos dão indicativos da religiosidade do casal que, possivelmente, dava ouvidos ao discurso da Igreja quanto à moderação na alimentação. Esta hipótese seria sustentada pelos seus corpos longilíneos e esguios.

A partir do século XIII o discurso hegemônico da religião passa a caminhar ao lado daquele médico, ofício que começava a passar pelo processo de profissionalização. Aparecem as universidades (Universidade de Bolonha, de Paris e de Montpellier), onde se discutiam principalmente as traduções das obras de Galeno, que entendia a dietética, por exemplo, como uso adequado das coisas não naturais: clima, exercícios físicos, banhos, sono, atividade sexual, emoções e dieta.

Vale ressaltar aqui que embora as ideias galênicas se constituíssem em cânone para a formação médica, assim como o modelo hipocrático, séculos mais tarde (XV-XVI) surgia o médico Paracelso que advogava a complementaridade entre os saberes herdeiros da alquimia e os saberes acadêmicos à época. Sem esquecermos, ainda, da própria filosofia que, segundo Jung, Paracelso compreendia “muito mais como vivência do que raciocínio”¹⁶ (p.19). A partir de Carl Gustav Jung, em *O Espírito na arte e na ciência*¹⁷, constatamos a singularidade das ideias deste importante alqui-

mista e médico quando Jung mostra que em seu pensamento a medicina deveria guiar-se não apenas em um foco medicamentoso e naquilo que era considerado o estritamente científico, mas, relacionar com os elementos da natureza e do cosmos.

Jung, por exemplo, destaca, em Paracelso, que o médico ao fazer diagnóstico, prognóstico, etiologia - assim como, a farmacologia e a manipulação dos medicamentos -, deve relacioná-los com os dados da astrologia. Percebemos, portanto, a singularidade de Paracelso no processo de compreensão das ciências médicas. Um saber mais amplo para a formação do médico e, por isso, muito contestado. Aliás, poderíamos dizer que tal singularidade também é percebida no próprio Jung, no que tange ao campo paradigmático da ciência hegemonicamente constituída por métodos cartesianos de separação entre objetividade e subjetividade ou entre sujeito e o objeto de sua investigação. Neste particular, certamente, não foi por acaso que Jung resolveu resgatar a figura de Paracelso, isto é, resgatar um saber rebelde de outro para também destacar o seu. A alquimia e a astrologia, constituíam - juntamente com o saber científico que se estabelecia neste período da história -, o discurso médico. Ou como diria Michel Foucault em *As palavras e as coisas*, uma *episteme* nascente à época.

Há neste período intensa produção de textos dietéticos, como *Le Régime Du corps* de Aldebrandin de Sienne, *Tacuinum Sanitatis* de Ibn Butlan e outros. Tais textos foram amplamente difundidos na Idade Média e estavam presentes nas casas das famílias aristocráticas, como era o caso do casal Arnolfini. As peças do mobiliário e o feito de encomendarem um retrato são indicativos do poder econômico do casal.

O discurso médico e o religioso eram aqueles que na época regiam em grande medida as escolhas dos indivíduos. Percebe-se que, mesmo neste contexto, não havia uma teoria dietética que unificasse a variedade de práticas alimentares¹⁷. O que podemos assegurar é que a quanti-

dade de informações - se pensarmos no terceiro polo da comunicação, o telemático informacional, enunciado por Pierre Lévy¹⁸, isto é, aquelas informações advindas dos meios de comunicação de massa, destacadamente, televisão e internet -, com as quais os indivíduos tinham de conviver não era tão ampla quanto a que nos deparamos hoje, onde a multiplicidade de vozes normativas em relação à alimentação parece resultar em voz alguma, inserindo-nos num cenário de mal-estar, incerteza e ansiedade. Uma anomia em direção à falta ou ao excesso.

A paródia de Botero, intitulada *Matrimonio Arnolfini segun van Eyck*, 1978, traz à tona corpos voluptuosos. Leituras podem ser feitas. Teria o uso destas formas apenas um caráter estético? Quando questionado sobre o motivo de pintar figuras obesas, Botero respondeu que não pintava pessoas gordas. O artista pontua seu estilo como estratégia estética e de formação de estilo, frustrando assim muitos que esperavam em seu discurso uma breve análise do lugar dos corpos obesos na sociedade atual. Evocaria ela à riqueza, à saúde e à sensualidade como até pouco tempo na América Latina era vista a gordura?¹⁹ Corpos voluptuosos? Corpos obesos?²⁰ A leitura da obra de arte, para além do propósito do autor depende também daquele que lê. Sendo assim, podemos chegar a múltiplas leituras e, numa delas, enxergar as formas na arte do colombiano Fernando Botero como uma referência ao excesso: ao excesso de complacência de um mundo que já não lida mais com regras, mas com uma cacofonia irritante, excesso de informações. "O excesso abafa a informação [...] enquanto a informação dá forma às coisas, a superinformação nos submerge no informe", diria Morin²¹ (p.21). Esse é o cenário no qual estão inseridos os Arnolfini de Botero. Os personagens, apesar de obesos, parecem estar prontos para flutuar devido à leveza do traço que pinta suas peles, traço marcante de uma sociedade fluida, onde a anomia assemelha-se mais à leveza que à solidez. Uma modernidade líquida? Os olhares e gestos não nos transmitem emoção. A obra de Botero é isenta de comentários emo-

cionais²². O excesso de complacência, fruto do excesso de discursos, gera corpos desmedidos: a sociedade do excesso. Através das lentes de alguém que tenta problematizar a crise na alimentação contemporânea este é o mundo do excesso que podemos encontrar em Botero.

É na brecha da anomia que florescem as pressões sobre o comensal moderno. A alimentação passa a constituir um campo de decisões pessoais que é alimentado por uma diversidade de normas que são dissonantes: higienistas, identitárias, hedonistas, estéticas²³. Há um rompimento da unanimidade de opiniões que arranca o sujeito da reprodução de normas costumeiras. Os comensais já não dispõem de conceitos reguladores. O corpo carrega estampada a marca deste mal-estar, que parece repousar sobre o excesso de informações. Recente pesquisa realizada por Claude Fischler, ao tentar investigar o que é comer bem para algumas populações de países distintos, constatou que ainda que os estadunidenses, mais que os europeus, relatem que hoje “é fácil encontrar qualquer informação da qual se necessite para comer de forma sadia”²⁴ (p.93), são eles os mesmos que acreditam que comem de forma menos saudável. Fischler, que cunhou o conceito de gastro-anomia, levanta a seguinte questão: “muita informação mata a informação?”²⁴ (p.94).

Em nossa sociedade a informação referente à alimentação assume formas assustadoras que alimentam a gastro-anomia. Nunca se produziu tanta informação com referência ao tema da comida. Embora a lista dos dez livros mais vendidos nos Estados Unidos da América (EUA) mude a cada semana, “dois tipos de publicação aparecem invariavelmente: obras de culinária que oferecem receitas de pratos cada vez mais refinados, deliciosos e sedutores e manuais de dieta prometendo regimes cada vez mais infalíveis para produzir corpos livres de gordura, esbeltos e graciosos”²⁵ (p.117). A vida dos comensais passa a ser uma experimentação contínua carregada de incertezas. Vivemos uma situação de “pânico alimentar”²⁵ (p.95).

Tão incômodo é o cenário que na mesma pesquisa de Claude Fischler²⁵ as pessoas da maior parte dos países onde foram realizadas as entrevistas demonstraram desejo de abrir mão da tarefa de escolher. Afastá-la-iam para não terem que lidar com o “fantasma da liberdade”? Durante o estudo, foram propostas duas questões às pessoas:

primeira: Você está com vontade de tomar um sorvete e tem de escolher entre duas sorveterias. A primeira oferece cinquenta sabores diferentes; a segunda, uma seleção de dez sabores. O preço é o mesmo, qual você escolheria?

segunda: Você foi convidado para jantar em um grande restaurante. O que prefere? Um cardápio com uma grande variedade de pratos ou um pequeno número de sugestões escolhidas pelo chef? (p.93).

De todos os países investigados apenas nos EUA as pessoas prefeririam o longo cardápio e a sorveteria com cinquenta sabores. Os outros optaram pela pré-seleção e por menos opções para lidarem.

Em seus contextos sociais, os Arnolfini de Botero teriam de lidar com uma quantidade de informações bem maior do que o casal de van Eyck teria de gerenciar. Além da multiplicidade de discursos, aqueles que ofereceriam um porto seguro frente às incertezas da anomia se apresentariam mais frouxos. Tomemos como exemplo o lugar da religião: comparativamente à Idade Média, hoje não cumpre um papel de estruturação hegemônica na sociedade²⁶. Não por coincidência, o tema apresenta-se timidamente na paródia do artista colombiano. O espelho rebuscado dá lugar a um simples espelho arredondado, fazendo desaparecer a referência às passagens bíblicas em seu redor. As laranjas, símbolo de pureza, dividem a cena com maçãs: fruto que a partir do século XIII, segundo Zierer passou a ser a principal representação do pecado de Adão e Eva no jardim do Éden²⁷. A senhora Arnolfini, rompendo com a “regra” cristã da castidade

aparece grávida no momento de celebração da boda. Na obra original, ainda que a noiva aparentemente ter o ventre um pouco abaulado, um olhar mais cuidadoso revela-nos que a senhora sim, parece apenas estar puxando a saia do vestido para a zona da barriga, o que causaria o volume na região. Essa era a moda da época. Imersos no excesso, teriam os Arnolfini de Botero de lidar com mais pontos de incerteza? Aparentemente, sim.

Pensar para, pensar com

Os quadros do colombiano Botero parecem um retrato dos corpos que vemos no espelho e nas ruas. Percebemos diariamente mergulhados num excesso desconcertante. A gastro-anomia instalou-se dentro de nós. Acompanha-nos desde as prateleiras do supermercado ao momento de levar o alimento à boca. A liberdade e a restrição, como nunca antes, travam a luta do equilíbrio no controle individual dos corpos. Carecemos de normas. De um discurso que nos diga por onde caminhar. O que comer? Como comer? Perguntas que ecoam diariamente na cabeça de nossa sociedade.

Quanto mais inseguros, mais vemos florescer a multiplicidade de discursos oferecidos pelo mercado para “ajudar” a lidar com nossos medos dietéticos: dieta da lua, de Atkins, de South Beach, do paleolítico, do mediterrâneo, *shakes* e chás que prometem perda de peso imediato e cobram caro por isso. E nós? Nós compramos.

O discurso da ciência da Nutrição é mais um dos milhares com que se depara o comensal contemporâneo em sua busca pelo “comer bem”. Ainda que esta seja uma mensagem que carrega o selo “científico”, paradigma hegemônico da sociedade atual, não esqueçamos que muito ainda há a se pensar. Qual é a linha entre norma e excesso? O que é saúde? O que é comer bem? Como diria Durkheim, o progresso de uma ciência pode ser percebido “pelo fato de as questões de que ela trata já não permanecerem estacionadas”⁴ (p.1). O que fazemos destas novas ques-

tões? Debruçamo-nos sobre elas? Pensamo-las junto aos comensais? Ou, ao contrário, nos limitamos a oferecer mais um discurso pronto às pessoas.

Devido ao sem fim de fatores que transpassam a alimentação, talvez não consigamos chegar a uma teoria alimentar que sintetize o bem comer. E, em verdade, esse talvez não devesse ser o propósito da Nutrição. O papel do nutricionista na sociedade atual deveria ser não o de pensar a alimentação *para* as pessoas - fornecendo-lhes fórmulas prontas para seus questionamentos - mas, pensar a alimentação *com* as pessoas, ajudando-as a lidar com a avalanche de informações com as quais se deparam, para que assim, possam constituir-se sujeitos de suas escolhas.

C O L A B O R A D O R E S

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Perinatal stress: Characteristics and effects on adult eating behavior

Estresse peri-natal: suas características e repercussões sobre o comportamento alimentar na vida adulta

Matilde Cesiana da SILVA¹

Ligia Cristina Monteiro GALINDO²

Julliet Araújo de SOUZA³

Raul Manhães de CASTRO²

Sandra Lopes de SOUZA⁴

ABSTRACT

Many studies have pointed out the importance of mother-child interaction in the early months of life. A few decades ago, a method called kangaroo care was developed and its main goal was to keep underweight or premature newborns in direct contact with the mother. This method has reduced the morbidity and mortality of these newborns, increasing their growth rate, breastfeeding time and mother-child contact. In rodents, the dam's presence is crucial for avoiding aggression factors that may trigger phenotypic adaptations in the pups with irreversible morphological, functional and behavioral consequences. Eating behavior is an adaptive response stemming from the external environment demand and modulated by opportunities and limitations imposed by the external environment. This behavior is regulated by a complex interaction of peripheral and central mechanisms that control hunger and satiety. The hypothalamus is a brain structure that integrates central and peripheral signals to regulate energy homeostasis and body weight. The hypothalamic nucleus have orexigenic peptides, such as neuropeptide Y and the Agouti-related peptide, and anorexigenic peptides, such as cocaine and amphetamine regulated transcript and proopiomelanocortin. An innovative study of eating behavior in experimental models of neonatal stress separates the mother from the offspring during lactation. This review describes the effects of stress during the neonatal period on general physiological factors, particularly on the control of eating behavior.

Indexing terms: Feeding behavior. Perinatal. Rats. Stress.

¹ Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Núcleo de Nutrição. Vitória de Santo Antão, PE, Brasil.

² Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Departamento de Anatomia. Av. Prof. Moraes Rego, s/n., Cidade Universitária, 50670-901, Recife, PE, Brasil. *Correspondência para/Correspondence to:* LCM GALINDO. *E-mail:* <sanlopesufpe@gmail.com>.

³ Universidade Federal de Pernambuco, Centro de Ciências da Saúde, Programa de Pós-Graduação em Nutrição. Recife, PE, Brasil.

⁴ Universidade Federal de Pernambuco, Centro de Ciências da Saúde, Departamento de Nutrição. Recife, PE, Brasil.

RESUMO

Muitos estudos têm apontado a importância da interação mãe-filho durante os primeiros meses de vida. Nas últimas décadas, foi desenvolvido um método, denominado mãe canguru, que tem como principal objetivo manter neonatos nascidos com baixo peso ou prematuros em contato direto com suas mães de forma contínua. Esse método tem reduzido a mortalidade e morbidade desses neonatos, aumentando medidas de crescimento, amamentação e contato mãe-filho. Em roedores, a presença da mãe é determinante para evitar a incidência de fatores agressores que possam desencadear adaptações fenotípicas dos filhotes com consequências morfofuncionais e comportamentais irreversíveis. O comportamento alimentar representa uma resposta adaptativa, decorrente da demanda do ambiente interno sendo modulado por oportunidades e limitações impostas pelo ambiente externo. Esse comportamento é regulado por uma interação complexa entre mecanismos periféricos e centrais que controlam a fome e a saciedade. O hipotálamo é a estrutura encefálica que integra sinais centrais e periféricos para regular a homeostase energética e o peso corporal. Nos núcleos hipotalâmicos são encontrados peptídeos orexigênicos como o neuropeptídeo Y e o peptídeo relacionado ao gene Agouti, e os anorexigênicos como o transcrito relacionado a cocaína e anfetamina e a pró-opiomelanocortina. O estudo do comportamento alimentar é inovador em modelos experimentais de estresse neonatal utilizando a separação entre mães e filhotes na fase de lactação. Esta revisão descreve os efeitos do estresse durante o período neonatal sobre aspectos fisiológicos gerais e particularmente sobre o controle do comportamento alimentar.

Termos de indexação: Comportamento alimentar. Perinatal. Ratos. Estresse.

INTRODUCTION

Many studies have pointed out the importance of the mother-child interaction during the first months of life for proper infant growth¹. Especially in cases of prematurity, this contact has proven effective against morphological and functional impairments in these newborns². Hence, a few decades ago a method called kangaroo care was developed. Its main objective is to keep low birth weight or premature newborns in direct contact with their mothers². This method has reduced the morbidity and mortality of these newborns, increasing their growth rate, breastfeeding time and the mother-child contact³. The morphological and functional adaptations to different durations and conditions of mother-child contact are known from studies with rodents influenced by contact. The presence of the dam is essential for avoiding the incidence of aggression factors that may trigger phenotypic adaptations in the pups with irreversible morphological, functional and behavioral consequences³. This characteristic is explored in experimental models of neonatal stress that separate the mother from the offspring during lactation⁴. Periodic maternal separation may cause malnutrition, thermal stress and damages to the

somatosensory contact⁵. One of the most common models involves repeated separation of the mother from the offspring (maternal separation model) and also of the offspring from the mother (maternal deprivation model) during lactation. These offspring show greater neuroendocrine responsiveness to stress in adulthood⁶, and greater vulnerability to depression, anxiety and eating disorders⁷, among others.

The objective of this review is to point out the repercussions of neonatal stress on the control mechanisms of eating behavior. Food intake and metabolism compose the two sides of the energy balance of an organism. Today, a high percentage of the global population has accumulated energy in the form of adipose tissue, which is considered a positive energy balance status, clinically defined as obesity. The great prevalence of obesity today is associated with genetic factors and with events that may have happened in early life. There are numerous accounts of eating and metabolic disorders in individuals who have been exposed to adverse environments in early life⁸. As an example, there are studies that show an important relationship between anorexia and bulimia nervosa in youth and sexual abuse during childhood. In animal studies, poor nutrition during

gestation and lactation promotes polyphagia and accumulation of body fat during adulthood. Studies of animal models of neonatal stress (maternal separation) found that in adulthood, these animals develop a preference for palatable foods, considered obesogenic. The identification of the damaging effects of maternal separation by experimental studies on eating behavior provides a base for clinical studies, helping to prevent eating disorders.

STRESS

The word stress was used for the first time in 1936 by the Hungarian physician Hans Selye in the publication "*What is stress?*". Known as general adaptation syndrome, stress is an unspecific response of the body to any external demand⁸. This response is divided into 3 stages: alarm (identification of the stressing agent), resistance (successfully fight off the stressing agent) and exhaustion (caused by harmful stress effects, the body can no longer respond to the stressing agent). The stressing agent is described as something that disrupts the body's homeostasis and thereby requires a physiological response⁸.

There are two classical stress response systems described in the literature: a) The neurovegetative system characterized by the release of adrenaline by the adrenal medulla; and b) The neuroendocrine system characterized by the release of glucocorticoids produced in the adrenal cortex in response to hypothalamic and hypophyseal stimuli. The acute activation of these systems is adaptive, promoting greater energy availability and blood to target organs. However, prolonged exposure to glucocorticoids stemming from the chronic activation of this system may be harmful⁹.

Any physical disturbance elicits a response to the stressing agent to maintain homeostasis and ease adaptation. In mammals, this response is triggered by the Hypothalamic-Pituitary-Adrenal Axis (HPA). The typical neuroendocrine response

to stress takes seconds: a) Increased secretion of catecholamines (adrenaline and noradrenaline) by the sympathetic nervous system; b) Activation of the Paraventricular Nucleus (PVN) of hypothalamus for expression of Arginine-Vasopressin (AVP) and Corticotropin-Releasing Hormone (CRH); and c) Increased Oxytocin (Oxt) secretion by the posterior pituitary. PVN neurons project to the median eminence, allowing the release of peptides from the hypophyseal portal system¹⁰. This results in the synthesis and release of many peptides with a common precursor, Proopiomelanocortin (POMC). Endogenous opioids, such as β -endorphin and Adrenocorticotropic Hormone (ACTH) are among these peptides¹¹. The HPA system that involves the release of glucocorticoid hormones by the adrenal gland (cortisol in humans and corticosterone in rats) is slow and persistent¹¹. A few minutes after an individual has been exposed to a stressing agent, plasma glucocorticoid levels increase, peaking between 30 and 60 minutes after the initial exposure. The CRH system is the initial factor in the neurochemical, behavioral and endocrine response to stress¹². The HPA axis, when stimulated by stress, activates CRH in the hypothalamus, promoting the release of ACTH by the anterior pituitary, which tells the adrenal gland to release glucocorticoids, such as corticosterone in rodents and cortisol in humans. Corticosterone has a negative feedback effect on the pituitary gland and hypothalamus, that is, it inhibits the release of ACTH and CRH¹². The glucocorticoid hormones are the final effectors of the HPA axis. These hormones promote homeostasis by mobilizing energy reserves, and catabolizing proteins, glycogen and fat. Furthermore, they improve cognitive function and inhibit the secretion of sex steroids. A sudden rise of glucocorticoid levels triggers a salutary adaptive response; however, high levels for long periods may harm the nervous system and other tissues and are associated with ventricular dilatation, cerebral atrophy, low cognitive capacity and possible neurotoxicity¹³. Corticosterone effects are mediated by two receptor subtypes: mineralo-

corticoid and glucocorticoid, with greater and smaller affinity for corticosterone, respectively¹³.

Mineralocorticoid receptors participate in the control of basal excitability and behavioral responsiveness, while glucocorticoid receptors are involved in terminating the stress response. These receptors may also act as ligand-dependent transcription factors. Mineralocorticoid receptors are found in some limbic areas, such as the hippocampus, while glucocorticoids are found in many brain regions, including the frontal cortex and PVN¹³. Glucocorticoid receptors have a key regulating role in the neuroendocrine control of the HPA axis and in the termination of the stress response. This occurs because it has a negative feedback effect on receptors of the hypothalamus and hypophysis, and in structures of the limbic system, such as the hippocampus and amygdala. Negative feedback operates in two modes: proactive, which involves the maintenance of baseline levels of the HPA axis, and reactive mode, which suppresses stress-induced ACTH and Corticosterone (CORT) levels¹³. The first mode determines sensitivity of the stress response or its threshold and involves functions of mineralocorticoid receptors with high affinity for corticosterone located in the higher brain regions. The second involves low-affinity glucocorticoid receptors located in PVN neurons, corticotropes, which are abundant in the cortical regions, hippocampus and ascending aminergic pathways, where they mediate the modulatory influence of corticosterone on HPA activity. The activation of mineralocorticoid receptors in the hippocampus inhibits activity of the HPA axis¹⁴. Hippocampal efferent projections activate GABAergic neurons located in the ventral lateral septum and Bed Nucleus of the Stria Terminalis (BNST), which project to neurons in the parvocellular region of the PVN¹⁴. The inhibition promoted by these hormones limits their action, which prevents catabolic, anti-reproductive and immunosuppressive effects on the body¹⁴.

The greater incidence of depression and other disorders in women is related to their

greater vulnerability to stress. The nervous system has gender-related specificities in the anatomic, metabolic and neurochemical levels, and in the responses to emotional stimuli. Clinical studies have found that the HPA axis has a function of sexual dysmorphism in normal or pathological conditions. Females have a greater baseline level of stress-induced cortisol and are more resistant to dexamethasone suppression of the HPA axis¹⁵.

Many studies with rats have found sex-related differences in HPA-axis function and in their responses to stressing situations or anxiety. These differences include greater responsiveness of the HPA axis, with stress-induced high plasma levels of the hormones ACTH and corticosterone in females. The amount of corticosterone secreted by females is not constant, it varies along the estral cycle¹⁶. Studies in humans suggest that steroid hormones are the main culprits in the gender-related differences associated with the HPA axis. Estrogen hinders the negative feedback of glucocorticoids, which enhances the stress response. It has been suggested that the greater sensitivity of the female HPA axis is related to estrogen-induced CRH transcription via receptors located in the PVN. However, note that gender-related differences in stress response are not limited to the HPA axis and circulating hormones, they are also found in the central neurotransmission systems¹⁷.

Events that occur during the rapid development of the nervous system, gestation and lactation in rats, and up to the third year of life in humans, are some of the factors that may change the body's response to stress. The periodic separation of dam and pups has been used as a model of perinatal stress. In adulthood, rats submitted to maternal separation demonstrate high anxiety, learning and memory deficits, high CRH expression in the PVN and hippocampus, and low glucocorticoid binding capacity to hippocampal receptors. Additionally, maternal separation modulates adult neurogenesis in the hippocampus¹⁸.

Stress during the neonatal period

During neonatal rodent development, there is a period of low responsiveness to stress (Stress Hyporesponsive period - SHRP), where the response of the adrenal gland to stress is minimum or inexistent. The main characteristics of this period are the high negative feedback effect of glucocorticoids on the hypothalamus and hypophysis, low adrenal sensitivity to ACTH and minimum corticosterone increase in response to most stressors. During the first two weeks after birth, the levels of corticosterone, ACTH and CRH in rodents are low¹⁶. This stage of HPA hyporesponsiveness may be an adaptive and protective mechanism, since high corticosterone levels during this critical period has harmful and catabolic effects, which include inhibition of brain growth, neuronal division, dendrite development and neuronal metabolism.

In the rat's brain, glucocorticoid receptors are functional by the 13th embryonic day, and the negative feedback of the HPA axis by corticosterone is established between the 13th and 17th days. In the early neonatal period, changes in the blood levels of corticosterone may affect the amount of these receptors¹⁹. The plasma levels of ACTH and corticosterone are high on the first day of life but they drop significantly from the second day on, and remain low during the first two weeks of life. The levels of these hormones will match those of adults on day 21, that is, around weaning time¹⁹. The immaturity of the adrenal gland of the developing rat is a consequence of the low synthesis and/or secretion of CRH and ACTH by the hypophysis during the first weeks of life. During the first two weeks of the rat's life, saturation of the pituitary glucocorticoid receptors may be associated with the highly inhibited ACTH release²⁰. During SHRP, free corticosterone circulation is high and may have access to tissues, especially corticotropic tissues, which may contribute to the high sensitivity of the feedback system during this period²¹. Moreover, glucocorticoid receptors may act at the suprahypophysial level to inhibit the synthesis and/or secretion of CRH and AVP. Low levels of

circulating glucocorticoids during the first two weeks of life seem to be essential for normal brain development. Glucocorticoids are important for normal brain maturation since they initiate terminal maturation, remodel axons and dendrites and impact cell survival. High glucocorticoid levels impair the development and function of the nervous system²². The administration of glucocorticoids in pregnant rats delays neuronal maturation and myelination, changing neuronal structure, synapse formation and neurogenesis²³.

Despite the existence of SHRP, many studies have shown neurochemical and behavioral changes that may persist throughout life when an individual is exposed to stressors during this period. Although hyporesponsive, these individuals have an acute response to the stress promoted by maternal separation, even when they are not exposed to any other stressor²⁴. During this phase, transcortin levels (a protein that transports glucocorticoids) are low and most glucocorticoids circulate in the plasma in an unbound state, that is, in a biologically active state²⁵. Therefore, despite the low total plasma concentration of glucocorticoids during SHRP, the concentration of their active form is high, which suffices for their biological actions. This must also be the mechanism responsible for the role played by stress in the programming of the nervous system and behavioral expression. In rats, at the end of the second week of life, the HPA axis matures, and there is a gradual increase in the baseline levels of corticosterone and stress responsiveness²⁶. There is a close relationship between HPA axis development, the environment and the establishment of adult behavioral patterns²⁶.

In rats, repeated maternal separation during the lactation period (neonatal stress model) promotes adulthood anxiety, learning and memory deficits²⁷, high expression of CRH in the PVN of the hypothalamus and hippocampus, and fewer glucocorticoid receptors²⁸. Variations in the care provided by dams to nursing pups affect gene regulation, neuronal number, neurotransmitter levels, and neuroendocrine and behavioral functions in response to stress. High dam care is

associated with low reactivity to stress in adult life, including low anxiety, HPA tonus and stress responsiveness²⁹. In humans, passive and active tactile stimulation of premature infants improve behavioral development, visceral function and maturation of the sympathetic and adrenal systems³⁰.

Neonatal stress and eating behavior

Eating behavior is an adaptive response stemming from the demand of the internal environment, modulated by opportunities and limitations imposed by the external environment. This behavior is regulated by a complex interaction between peripheral and central mechanisms that control hunger and satiety³¹. The hypothalamus is the brain structure that integrates central and peripheral signals to regulate energy homeostasis and body weight³². The hypothalamic nuclei contain orexigenic peptides, such as Neuropeptide Y (NPY) and Agouti-related Peptide (AgRP), and anorexigenic peptides, such as Cocaine and Amphetamine Regulated Transcript (CART) and Proopiomelanocortin (POMC)³³. Many peripheral substances act on the neurons that produce these peptides to influence eating behavior³³. Among these are the hormones involved in the stress response.

Stress promotes important adjustments to energy metabolism. Individuals exposed to stressful situations have low food intake and body weight and high energy expenditure³⁴. However, when food is energy-dense or palatable, stress promotes high intake³⁵. When submitted to a test of food preferences, stressed individuals prefer fatty foods³⁶. Glucocorticoids may be responsible for the preference for palatable foods. The removal of glucocorticoids by adrenalectomy reduces food intake by 10% to 20%, slowing weight gain³⁷.

Neonatal stress adjusts eating behavior in response to different demands during adulthood, since HPA axis dysfunction is related to the pathogenesis of eating disorders³⁸. Rats that were separated from their dams are more vulnerable

to the development of anorexia nervosa. Individuals with eating disorders are usually survivors of childhood abuse, and HPA dysfunction is implicated in this pathophysiology³⁹. Many patients with eating disorders report being childhood-abuse survivors. A study with patients with bulimia nervosa found that many of their parents were separated, divorced or widowed⁴⁰. Another study reported that patients with bulimia nervosa were more likely to have been sexually abused during childhood than patients with anorexia nervosa⁴⁰.

Not many studies of experimental models of neonatal stress describe its repercussions on the mechanisms that control eating behavior. In rats, food intake during adulthood was not affected by maternal separation. However, when these animals were submitted to food restriction cycles, they presented rebound polyphagia⁴¹. Being separated from the dam promoted high food intake in adolescent rats that were exposed to successive cycles of food restriction and abundance, possibly because of the increased responsiveness of the HPA axis. Rats are considered adolescents at 60 days of age, when they reach sexual maturity⁴².

Glucocorticoids are involved in the regulation of energy balance and affects hypothalamic neuropeptides⁴³. Neurons that express NPY in the hypothalamic arcuate nucleus have glucocorticoid receptors. High plasma levels of corticosterone may be necessary for increasing the expression of messenger Ribonucleic Acid (mRNA) NPY⁴⁴.

Animals that survived perinatal malnutrition, a type of stress, have polyphagia associated with high levels of hypothalamic peptides that stimulate hunger (NPY) and low levels of those that inhibit it (POMC)⁴⁵, as well as changes in the serotonergic control of satiety⁴⁶. On the one hand, maternal separation increases the baseline levels of hypothalamic NPY⁴⁷; on the other, it inhibits this increase in response to fasting⁴⁸. The baseline levels of anorexigenic hypothalamic peptides, such as POMC and CART, and orexigenic peptides, such as NPY, are not changed by maternal separation

of young female rats⁴⁹. However, when these female rats are submitted to a 48-hour fast, maternal separation affected the expression of these neuropeptides, with an increase in NPY and reduction in POMC and CART⁵⁰. This review indicates that different factors involved in the mother-child interaction have distinct programming influences on adult eating behavior.

CONTRIBUTORS

MC SILVA database search and article writing;
SL SOUZA article writing and review; LCM GALINDO,
RM CASTRO and JA SOUZA final article review.

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Preparo do manuscrito

Submissão de trabalhos

Serão aceitos trabalhos acompanhados de carta assinada por todos os autores, com descrição do tipo de trabalho e da área temática, declaração de que o trabalho está sendo submetido apenas à Revista de Nutrição e de concordância com a cessão de direitos autorais e uma carta sobre a principal contribuição do estudo para a área.

Caso haja utilização de figuras ou tabelas publicadas em outras fontes, deve-se anexar documento que ateste a permissão para seu uso.

Enviar os manuscritos via *site* <<http://www.scielo.br/rn>>, preparados em espaço entrelinhas 1,5, com fonte *Arial* 11. O arquivo deverá ser gravado em editor de texto similar ou superior à versão 97-2003 do *Word* (*Windows*).

É fundamental que o escopo do artigo **não contenha qualquer forma de identificação da autoria**, o que inclui referência a trabalhos anteriores do(s) autor(es), da instituição de origem, por exemplo.

O texto deverá contemplar o número de palavras de acordo com a categoria do artigo. As folhas deverão ter numeração personalizada desde a folha de rosto (que deverá apresentar o número 1). O papel deverá ser de tamanho A4, com formatação de margens superior e inferior (no mínimo 2,5cm), esquerda e direita (no mínimo 3cm).

Os artigos devem ter, aproximadamente, 30 referências, exceto no caso de artigos de revisão, que podem apresentar em torno de 50. Sempre que uma referência possuir o número de *Digital Object Identifier* (DOI), este deve ser informado.

Versão reformulada: a versão reformulada deverá ser encaminhada via <<http://www.scielo.br/rn>>. **O(s) autor(es) deverá(ão) enviar apenas a última versão do trabalho.**

O texto do artigo deverá empregar fonte colorida (cor azul) ou sublinhar, para todas as alterações, juntamente com uma carta ao editor, reiterando o interesse em publicar nesta Revista e informando quais alterações foram processadas no manuscrito. Se houver discordância quanto às recomendações dos revisores, o(s) autor(es) deverão apresentar os argumentos que justificam sua posição.

O título e o código do manuscrito deverão ser especificados.

Página de rosto deve conter

a) título completo - deve ser conciso, evitando excesso de palavras, como "avaliação do...", "considerações acerca de..." "estudo exploratório...";

b) *short title* com até 40 caracteres (incluindo espaços), em português (ou espanhol) e inglês;

c) nome de todos os autores por extenso, indicando a filiação institucional de cada um. Será aceita uma única titulação e filiação por autor. O(s) autor(es) deverá(ão), portanto, escolher, entre suas titulações e filiações institucionais, aquela que julgar(em) a mais importante;

d) todos os dados da titulação e da filiação deverão ser apresentados por extenso, sem siglas;

e) indicação dos endereços completos de todas as universidades às quais estão vinculados os autores;

f) indicação de endereço para correspondência com o autor para a tramitação do original, incluindo fax, telefone e endereço eletrônico.

Observação: esta deverá ser a única parte do texto com a identificação dos autores.

Resumo: todos os artigos submetidos em português ou espanhol deverão ter resumo no idioma original e em inglês, com um mínimo de 150 palavras e máximo de 250 palavras.

Os artigos submetidos em inglês deverão vir acompanhados de resumo em português, além do *abstract* em inglês.

Para os artigos originais, os resumos devem ser estruturados destacando objetivos, métodos básicos adotados, informação sobre o local, população e amostragem da pesquisa, resultados e conclusões mais relevantes, considerando os objetivos do trabalho, e indicando formas de continuidade do estudo.

Para as demais categorias, o formato dos resumos deve ser o narrativo, mas com as mesmas informações.

O texto não deve conter citações e abreviaturas. Destacar no mínimo três e no máximo seis termos de indexação, utilizando os descritores em Ciência da Saúde - DeCS - da Bireme <<http://decs.bvs.br>>.

Texto: com exceção dos manuscritos apresentados como Revisão, Comunicação, Nota Científica e Ensaio, os trabalhos deverão seguir a estrutura formal para trabalhos científicos:

Introdução: deve conter revisão da literatura atualizada e pertinente ao tema, adequada à apresentação

do problema, e que destaque sua relevância. Não deve ser extensa, a não ser em manuscritos submetidos como Artigo de Revisão.

Métodos: deve conter descrição clara e sucinta do método empregado, acompanhada da correspondente citação bibliográfica, incluindo: procedimentos adotados; universo e amostra; instrumentos de medida e, se aplicável, método de validação; tratamento estatístico.

Em relação à análise estatística, os autores devem demonstrar que os procedimentos utilizados foram não somente apropriados para testar as hipóteses do estudo, mas também corretamente interpretados. Os níveis de significância estatística (ex. $p < 0,05$; $p < 0,01$; $p < 0,001$) devem ser mencionados.

Informar que a pesquisa foi aprovada por Comitê de Ética credenciado junto ao Conselho Nacional de Saúde e fornecer o número do processo.

Ao relatar experimentos com animais, indicar se as diretrizes de conselhos de pesquisa institucionais ou nacionais - ou se qualquer lei nacional relativa aos cuidados e ao uso de animais de laboratório - foram seguidas.

Resultados: sempre que possível, os resultados devem ser apresentados em tabelas ou figuras, elaboradas de forma a serem auto-explicativas e com análise estatística. Evitar repetir dados no texto.

Tabelas, quadros e figuras devem ser limitados a cinco no conjunto e numerados consecutiva e independentemente com algarismos arábicos, de acordo com a ordem de menção dos dados, e devem vir em folhas individuais e separadas, com indicação de sua localização no texto. **É imprescindível a informação do local e ano do estudo.** A cada um se deve atribuir um título breve. Os quadros e tabelas terão as bordas laterais abertas.

O(s) autor(es) se responsabiliza(m) pela qualidade das figuras (desenhos, ilustrações, tabelas, quadros e gráficos), que deverão ser elaboradas em tamanhos de uma ou duas colunas (7 e 15cm, respectivamente); **não é permitido o formato paisagem.** Figuras digitalizadas deverão ter extensão jpeg e resolução mínima de 400 dpi.

Gráficos e desenhos deverão ser gerados em programas de desenho vetorial (*Microsoft Excel, CorelDraw, Adobe Illustrator* etc.), acompanhados de seus parâmetros quantitativos, em forma de tabela e com nome de todas as variáveis.

A publicação de imagens coloridas, após avaliação da viabilidade técnica de sua reprodução, será custeada pelo(s) autor(es). Em caso de manifestação de interesse por parte do(s) autor(es), a Revista de Nutrição providen-

ciará um orçamento dos custos envolvidos, que poderão variar de acordo com o número de imagens, sua distribuição em páginas diferentes e a publicação concomitante de material em cores por parte de outro(s) autor(es).

Uma vez apresentado ao(s) autor(es) o orçamento dos custos correspondentes ao material de seu interesse, este(s) deverá(ão) efetuar depósito bancário. As informações para o depósito serão fornecidas oportunamente.

Discussão: deve explorar, adequada e objetivamente, os resultados, discutidos à luz de outras observações já registradas na literatura.

Conclusão: apresentar as conclusões relevantes, considerando os objetivos do trabalho, e indicar formas de continuidade do estudo. **Não serão aceitas citações bibliográficas nesta seção.**

Agradecimentos: podem ser registrados agradecimentos, em parágrafo não superior a três linhas, dirigidos a instituições ou indivíduos que prestaram efetiva colaboração para o trabalho.

Anexos: deverão ser incluídos apenas quando imprescindíveis à compreensão do texto. Caberá aos editores julgar a necessidade de sua publicação.

Abreviaturas e siglas: deverão ser utilizadas de forma padronizada, restringindo-se apenas àquelas usadas convencionalmente ou sancionadas pelo uso, acompanhadas do significado, por extenso, quando da primeira citação no texto. Não devem ser usadas no título e no resumo.

Referências de acordo com o estilo Vancouver

Referências: devem ser numeradas consecutivamente, seguindo a ordem em que foram mencionadas pela primeira vez no texto, conforme o estilo Vancouver.

Nas referências com dois até o limite de seis autores, citam-se todos os autores; acima de seis autores, citam-se os seis primeiros autores, seguido de *et al.*

As abreviaturas dos títulos dos periódicos citados deverão estar de acordo com o *Index Medicus*.

Não serão aceitas citações/referências de monografias de conclusão de curso de graduação, **de trabalhos** de Congressos, Simpósios, *Workshops*, Encontros, entre outros, e **de textos não publicados** (aulas, entre outros).

Se um trabalho não publicado, de autoria de um dos autores do manuscrito, for citado (ou seja, um artigo *in press*), será necessário incluir a carta de aceitação da revista que publicará o referido artigo.

Se dados não publicados obtidos por outros pesquisadores forem citados pelo manuscrito, será necessário incluir uma carta de autorização, do uso dos mesmos por seus autores.

Citações bibliográficas no texto: deverão ser expostas em ordem numérica, em algarismos arábicos, meia linha acima e após a citação, e devem constar da lista de referências. Se forem dois autores, citam-se ambos ligados pelo "&"; se forem mais de dois, cita-se o primeiro autor, seguido da expressão *et al.*

A exatidão e a adequação das referências a trabalhos que tenham sido consultados e mencionados no texto do artigo são de responsabilidade do autor. Todos os autores cujos trabalhos forem citados no texto deverão ser listados na seção de Referências.

Exemplos

Artigo com um autor

Burlandy L. A construção da política de segurança alimentar e nutricional no Brasil: estratégias e desafios para a promoção da intersetorialidade no âmbito federal de governo. *Ciênc Saúde Coletiva*. 2009; 14(3):851-60. doi: 10.1590/S1413-81232009000300020.

Artigo com mais de seis autores

Oliveira JS, Lira PIC, Veras ICL, Maia SR, Lemos MCC, Andrade SLL, *et al.* Estado nutricional e insegurança alimentar de adolescentes e adultos em duas localidades de baixo índice de desenvolvimento humano. *Rev Nutr*. 2009; 22(4): 453-66. doi: 10.1590/S1415-52732009000400002.

Livro

Alberts B, Lewis J, Raff MC. *Biologia molecular da célula*. 5ª ed. Porto Alegre: Artmed; 2010.

Capítulos de livros

Aciolly E. Banco de leite. In Aciolly E. *Nutrição em obstetria e pediatria*. 2ª ed. Rio de Janeiro: Guanabara Koogan; 2009. Unidade 4.

Dissertações e teses

Duran ACFL. *Qualidade da dieta de adultos vivendo com HIV/AIDS e seus fatores associados* [mestrado]. São Paulo: Universidade de São Paulo; 2009.

Artigo em suporte eletrônico

Sichieri R, Moura EC. Análise multinível das variações no índice de massa corporal entre adultos, Brasil, 2006. *Rev Saúde Pública*. 2009 [acesso 2009 dez 18]; 43(Supl 2): 90-7. Disponível em: <<http://www.scielo.br/scielo.php>

script=sci_arttext&pid=S0034-891020090000900012&lng=pt&nrm=iso>. doi: 10.1590/S0034-89102009000900012.

Livro em suporte eletrônico

Brasil. Alimentação saudável para pessoa idosa: um manual para o profissional da saúde. Brasília: Ministério da Saúde; 2009 [acesso 2010 jan 13]. Disponível em: <http://200.18.252.57/services/e-books/alimentacao_saudavel_idosa_profissionais_saude.pdf>.

Capítulo de livro em suporte eletrônico

Emergency contraceptive pills (ECPs). In World Health Organization. Medical eligibility criteria for contraceptive use. 4th ed. Geneva: WHO; 2009 [cited 2010 Jan 14]. Available from: <http://whqlibdoc.who.int/publications/2009/9789241563888_eng.pdf>.

Texto em formato eletrônico

Sociedade Brasileira de Nutrição Parental e Enteral. Assuntos de interesse do farmacêutico atuante na terapia nutricional. 2008/2009 [acesso 2010 jan 14]. Disponível em: <<http://www.sbnpe.com.br/ctdpg.php?pg=13&ct=A>>.

Para outros exemplos recomendamos consultar as normas do *Committee of Medical Journals Editors* (Grupo Vancouver) <<http://www.icmje.org>>.

Lista de checagem

- Declaração de responsabilidade e transferência de direitos autorais assinada por cada autor.

- Verificar se o texto, incluindo resumos, tabelas e referências, está reproduzido com letras fonte *Arial*, corpo 11 e entrelinhas 1,5 e com formatação de margens superior e inferior (no mínimo 2,5cm), esquerda e direita (no mínimo 3cm).

- Indicação da categoria e área temática do artigo.

- Verificar se estão completas as informações de legendas das figuras e tabelas.

- Preparar página de rosto com as informações solicitadas.

- Incluir o nome de agências financiadoras e o número do processo.

- Indicar se o artigo é baseado em tese/dissertação, colocando o título, o nome da instituição, o ano de defesa.

- Incluir título do manuscrito, em português e em inglês.

- Incluir título abreviado (*short title*), com 40 caracteres, para fins de legenda em todas as páginas.

- Incluir resumos estruturados para trabalhos submetidos na categoria de originais e narrativos para manuscritos submetidos nas demais categorias, com um número de 150 palavras e no máximo 250 palavras nos dois idiomas, português e inglês, ou em espanhol, nos casos em que se aplique, com termos de indexação.

- Verificar se as referências estão normalizadas segundo estilo *Vancouver*, ordenadas na ordem em que foram mencionadas pela primeira vez no texto, e se todas estão citadas no texto.

- Incluir permissão de editores para reprodução de figuras ou tabelas publicadas.

- Cópia do parecer do Comitê de Ética em pesquisa.

Documentos

Declaração de responsabilidade e transferência de direitos autorais

Cada autor deve ler e assinar os documentos (1) Declaração de Responsabilidade e (2) Transferência de Direitos Autorais, nos quais constarão:

- Título do manuscrito:

- Nome por extenso dos autores (na mesma ordem em que aparecem no manuscrito).

- Autor responsável pelas negociações:

1. Declaração de responsabilidade: todas as pessoas relacionadas como autoras devem assinar declarações de responsabilidade nos termos abaixo:

- “Certifico que participei da concepção do trabalho para tornar pública minha responsabilidade pelo seu conteúdo, que não omiti quaisquer ligações ou acordos de financiamento entre os autores e companhias que possam ter interesse na publicação deste artigo”.

- “Certifico que o manuscrito é original e que o trabalho, em parte ou na íntegra, ou qualquer outro trabalho com conteúdo substancialmente similar, de minha autoria, não foi enviado a outra Revista e não o será, enquanto sua publicação estiver sendo considerada pela Revista de Nutrição, quer seja no formato impresso ou no eletrônico”.

2. Transferência de Direitos Autorais: “Declaro que, em caso de aceitação do artigo, a Revista de Nutrição passa a ter os direitos autorais a ele referentes, que se tornarão propriedade exclusiva da Revista, vedado a qualquer

reprodução, total ou parcial, em qualquer outra parte ou meio de divulgação, impressa ou eletrônica, sem que a prévia e necessária autorização seja solicitada e, se obtida, farei constar o competente agradecimento à Revista”.

Assinatura do(s) autores(s) Data ____/____/____

Justificativa do artigo

Destaco que a principal contribuição do estudo para a área em que se insere é a seguinte: _____

(Escreva um parágrafo justificando porque a revista deve publicar o seu artigo, destacando a sua relevância científica, a sua contribuição para as discussões na área em que se insere, o(s) ponto(s) que caracteriza(m) a sua originalidade e o conseqüente potencial de ser citado)

Dada a competência na área do estudo, indico o nome dos seguintes pesquisadores (três) que podem atuar como revisores do manuscrito. Declaro igualmente não haver qualquer conflito de interesses para esta indicação.

Toda correspondência deve ser enviada à Revista de Nutrição no endereço abaixo

Núcleo de Editoração SBI - *Campus II*

Av. John Boyd Dunlop, s/n., Prédio de Odontologia, Jd. Ipaussurama, 13060-904, Campinas, SP, Brasil.

Fone/Fax: +55-19-3343-6875

E-mail: sbi.submissionrn@puc-campinas.edu.br

Web: <http://www.scielo.br/rn>

GUIDE FOR AUTHORS

Scope and policy

The **Brazilian Journal of Nutrition** is a specialized periodical that publishes articles that contribute to the study of Nutrition in its many sub-areas and interfaces. It is published bimonthly and open to contributions of the national and international scientific communities.

Submitted manuscripts may be rejected without detailed comments after initial review by at least two **Brazilian Journal of Nutrition** editors if the manuscripts are considered inappropriate or of insufficient scientific priority for publication in the Journal.

The Board of Editors does not assume responsibility for concepts and illustrations emitted in signed articles.

Article category

The Journal accepts unpublished articles in Portuguese, Spanish or English, with title, abstract and keywords in the original language and in English, in the following categories:

Original: contributions that aim to disclose the results of unpublished researches, taking into account the relevance of the theme, the scope and the knowledge generated for the research area (maximum limit of 5 thousand words).

Special: invited articles on current themes (maximum limit of 6 thousand words).

Review (by invitation): synthesis of the knowledge available on a given theme, based on analysis and interpretation of the pertinent literature, aiming to make a critical and comparative analysis of the works in the area and discuss the methodological limitations and its scope. It also allows the indication of perspectives of continuing studies in that line of research (maximum limit of 6 thousand words). There will be a maximum of two reviews per issue.

Communication: information reported on relevant themes and based on recent research, whose objective is to subsidize the work of professionals who work in the field, serving as a presentation or update on the theme (maximum limit of 4 thousand words).

Scientific note: partial unpublished data of an ongoing research (maximum limit of 4 thousand words).

Essay: works that can bring reflection and discussion of a subject that generates questioning and hypotheses for future research (maximum limit of 5 thousand words).

Thematic Section (by invitation): section whose aim is to publish 2 or 3 coordinated articles from different authors covering a theme of current interest (maximum of 10 thousand words).

Article's category and subject area

Authors should indicate the article's category and subject area, namely: food and social sciences, nutritional assessment, nutritional biochemistry, nutrition, nutrition education, epidemiology and statistics, micronutrients, clinical nutrition, experimental nutrition, nutrition and geriatrics, nutrition, maternal and infant nutrition in meal production, food and nutrition policies and health.

Research involving living beings

Results of research involving human beings and animals, must contain a copy of the Research Ethics Committee approval.

Registration of Clinical Trials

Articles with results of clinical researches must present an identification number in one of the Register of Clinical Trials validated by criteria established by the World Health Organization (WHO) and International Committee of Medical Journal Editors (ICMJE), whose addresses are available at the ICMJE site. The identification number must be included at the end of the abstract.

The authors must indicate three possible reviewers for the manuscript. Alternatively, the authors may indicate three reviewers to whom they do not want their manuscript to be sent.

Editorial procedures

Authorship

The list of authors, included below the title, should be limited to 6. The authorship credit must be based on substantial contributions, such as conception and design, or analysis and interpretation of the data. The inclusion of authors whose contribution does not include the criteria mentioned above is not justified.

The manuscripts must explicitly contain in the identification page the contribution of each one of the authors.

Manuscript judgment process

All manuscripts will only start undergoing the publication process if they are in agreement with the Instructions to the Authors. If not, **they will be returned for the authors to make the appropriate adjustments**, include a letter or other documents that may be necessary.

It is strongly recommended that the author(s) seek professional language services (reviewers and/or translators certified in the Portuguese or English languages) before they submit articles that may have semantic, grammar, syntactic, morphological, idiomatic or stylistic mistakes. The authors must also avoid using the first person of the singular, "my study...", or the first person of the plural "we noticed...", since scientific texts ask for an impersonal, non-judgmental discourse.

Articles with any of the mistakes mentioned above **will be returned even before they are submitted to assessment** regarding the merit of the work and the convenience of its publication.

Pre-evaluation: Scientific Editors evaluate manuscripts according to their originality, application, academic quality and relevance in nutrition.

Once the articles are approved in this phase, they will be sent to *ad hoc* peer reviewers selected by the editors. Each manuscript will be sent to two reviewers of known competence in the selected theme. One of them may be chosen by the authors' indication. If there is disagreement, the manuscript will be sent to a third reviewer.

The entire manuscript process will end on the second version, which will be final.

The peer review process used is the blind review, where the identity of the authors and the reviewers is not mutually known. Thus the authors must do everything possible to avoid the identification of the authors of the manuscript.

The opinions of the reviewers are one of the following: a) approved; b) new analysis needed; c) refused. The authors will always be informed of the reviewers' opinion.

Reviews are examined by the Editors who will recommend or not the manuscript's approval by the Scientific Editor.

Rejected manuscripts that can potentially be reworked can be resubmitted as a new article and will undergo a new peer review process.

Conflict of interest

If there are conflicts of interest regarding the reviewers, the Editorial Committee will send the manuscript to another *ad hoc* reviewer.

Accepted manuscripts: accepted manuscripts may return to the authors for the approval of changes done in the editorial and normalization process, according to the Journal's style.

Proof sheets: the proof sheets will be sent to the authors for correction of printing mistakes. The proof sheets need to be sent back to the Editorial Center within the stipulated deadline. Other changes to the manuscript will not be accepted during this phase.

Preparation of the manuscript

Submission of works

Manuscripts need to be accompanied by a letter signed by all the authors describing the type of work and the thematic area, a declaration that the manuscript is being submitted only to the Journal of Nutrition, an agreement to transfer the copy rights and a letter stating the main contribution of the study to the area.

If the manuscript contains figures or tables that have already been published elsewhere, a document given by the original publisher authorizing their use must be included.

The manuscripts need to be sent to the Editorial Center of the Journal, to the site <<http://www.scielo.br/rn>> with a line spacing of 1.5, font Arial 11. The file must be in Microsoft Word (doc) format version 97-2003 or better.

It is essential that the body of the article **does not contain any information that may identify the author(s)**, including, for example, reference to previous works of the author(s) or mention of the institution where the work was done.

The articles should have approximately 30 references, except for review articles, which may contain about 50 references. A reference must always contain the Digital Object Identifier (DOI).

Reviewed version: send the copies of the reviewed version to the site <<http://www.scielo.br/rn>>. **The author(s) must send only the last version of the work.**

Please use a color font (preferably blue) or underline all the changes made to the text, Include a letter to the editor confirming your interest in publishing your article in this Journal and state which changes were made in the manuscript. If the authors disagree with the opinion of the reviewers, they should present arguments that justify their position. The title and the code of the manuscript must be specified.

Title page must contain

a) full title - must be concise, avoiding excess wording, such as "assessment of...", "considerations on...", "exploratory study...";

b) short title with up to 40 characters (including spaces) in Portuguese (or Spanish) and English;

c) full name of all the authors, indicating the institutional affiliation of each one of them. Only one title

and affiliation will be accepted per author. The author(s) should therefore choose among their titles and institutional affiliations those that they deem more important;

d) all data of the titles and affiliations must not contain any abbreviations;

e) provide the full address of all the universities to which the authors are affiliated;

f) provide the full address for correspondence of the main author for the editorial procedures, including fax and telephone numbers and e-mail address.

Observation: this must be the only part of the text with author identification.

Abstract: all articles submitted in Portuguese or Spanish must contain an abstract in the original language and in English, with at least 150 words and at most 250 words.

The articles submitted in English must contain an abstract in Portuguese in addition to the abstract in English.

Original articles must contain structured abstracts containing objectives, basic research methods, information regarding study location, population and sample, results and most relevant conclusions, considering the objectives of the work and indicating ways of continuing the study.

The other categories should contain a narrative abstract but with the same information.

The text should not contain citations and abbreviations. Provide from 3 to 6 keywords using Bireme's Health Sciences descriptors. <<http://decs.bvs.br>>.

Text: except for the manuscripts presented as Review, Communication, Scientific Note and Assay, the works must follow the formal structure for scientific works:

Introduction: must contain a current literature review pertinent to the theme and appropriate to the presentation of the problem, also emphasizing its relevance. It should not be extensive except for manuscripts submitted as Review Articles.

Methods: must contain a clear and brief description of the method, including the corresponding literature: procedures, universe and sample, measurement tools, and validation method and statistical treatment when applicable.

Regarding the statistical analysis, the authors should demonstrate that the procedures were not only appropriate to test the hypotheses of the study but were also interpreted correctly. The statistical significance levels (e.g. $p < 0.05$; $p < 0.01$; $p < 0.001$) must be mentioned.

Inform that the research was approved by an Ethics Committee certified by the National Council of Health and provide the number of the protocol.

When experiments with animals are reported, indicate if the guidelines of the institutional or national research councils - or if any national law regarding the care and use of laboratory animals - were followed.

Results: whenever possible, the results must be presented in self-explanatory tables and figures and contain statistical analysis. Avoid repeating the data in the text.

Tables, charts and figures should be limited to five in all and given consecutive and independent numbers in Arabic numerals, according to the order the data is mentioned, and should be presented in individual sheets and separated, indicating their location in the text. **It is essential to inform the location and year of the study.** Each one should have a brief title. The charts and tables must be open laterally.

The author(s) are responsible for the quality of the figures (drawings, illustrations, tables and graphs) that should be large enough to fit one or two columns (7 and 15cm respectively); **the landscape format is not accepted.** Figures should be in jpeg format and have a minimum resolution of 400 dpi.

Graphs and drawings should be made in vector design software (Microsoft Excel, CorelDraw, Adobe Illustrator etc.), followed by their quantitative parameters in a table and the name of all its variables.

The publication of color images will be paid by the author(s) once the technical viability of their reproduction is verified. If the authors are interested, the Journal will provide the costs which will vary according to the number of images, their distribution in different pages, and the concomitant publication of color material by other author(s).

Once the authors are informed of such costs, they are expected to pay via wire transfer. The information for the wire transfer will be given at the appropriate time.

Discussion: the discussion must properly and objectively explore the results under the light of other observations already published in the literature.

Conclusion: present the relevant conclusions, considering the objectives of the work, and indicate ways to continue the study. **Literature citations will not be accepted in this section.**

Acknowledgments: may be made in a paragraph no bigger than three lines to institutions or individuals who actually collaborated with the work.

Attachments: should be included only when they are essential to the understanding of the text. The editors will decide upon the need of their publication.

Abbreviations and acronyms: should be used in a standardized fashion and restricted to those used conventionally or sanctioned by use, followed by the meaning in full when it is first mentioned in the text. They must not be used in the title and abstract.

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Alberts B, Lewis J, Raff MC. *Biologia molecular da célula*. 5ª ed. Porto Alegre: Artmed; 2010.

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Dissertations and theses

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