REVISTA DE NUTRIÇÃO
Brazilian Journal of Nutrition

ORIGINAL
ORIGINAL

Epidemiology and Statistics
Epidemiologia e Estatística

Editor
Luciana Bertoldi Nucci

Conflict of interest
The authors declare that they have no conflicts of interest.

Received
September 29, 2022

Final version
August 22, 2023

Approved
October 26, 2023

The epidemiological burden of reducing salt intake in Paraguay: A modeling study

A carga epidemiológica da redução do sal no Paraguai: um estudo de modelagem

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How to cite this article: Burgos R, Santacruz E, Duarte-Zoilan D, Turnes C, Benitez G, Nilson EAF. The epidemiological burden of reducing salt intake in Paraguay: A modeling study. Rev Nutr. 2023;36:e220216. https://doi.org/10.1590/1678-9865202336e220216

ABSTRACT

Objective
This study aims to estimate the epidemiological burden of excessive salt intake reduction and achieve the World Health Organization salt reduction target for 2025 in Paraguay, in 2019.

Methods
We used the Preventable Risk Integrated Model, a comparative risk assessment macro-simulation model, to estimate the averted deaths, disease incidence, and disability-adjusted life years from cardiovascular disease attributable to salt intake in the population of Paraguay for different salt reduction policy scenarios.

Results
As a result, in Paraguay, excessive salt intake (over 5 g/day) is responsible for approximately 2,656 cardiovascular disease deaths (95% Uncertainty Interval: 1,250-3,765), 4,816 cardiovascular disease cases (95% UI: 2,251-6,947), and 60,529 disability-adjusted life years (95% UI: 27,828-86,258) per year. By reducing salt consumption by 30%, as recommended by the World Health Organization until 2025, approximately 1,188 deaths (95% UI: 520 to 1,820), 2,100 incident cases (95% UI: 923-3,234), and 27,272 disability-adjusted life years (95% UI: 11,999-41,675) from cardiovascular disease could be averted every year.

Conclusion
In conclusion, the burden of cardiovascular disease attributable to excessive salt intake is significant and salt reduction policies must become a priority in Paraguay.

Keywords: Salt. Sodium. Cardiovascular disease. Mortality. Hypertension. Non-communicable disease. Paraguay.

RESUMO

Objetivo

Este estudo visa estimar a carga epidemiológica do consumo excessivo de sal e o alcance da meta da Organização Mundial de Saúde para 2025 no Paraguai, em 2019.

Métodos

*Foi usado um modelo de avaliação comparativa de risco (*Preventable Risk Integrated Model*) para análise comparativa de risco para estimar mortes, casos incidentes e anos de vida ajustados por incapacidade (DALYs) por doenças cardiovasculares atribuíveis ao consumo excessivo de sal na população paraguaia em diferentes cenários.*

Resultados

No Paraguai, o consumo excessivo de sal é responsável por aproximadamente 2.656 mortes (Intervalo de Incerteza 95%: 1.250-3.765), 4.816 casos incidentes (95% II: 2.251-6.947) e 60.529 DALYs (95% II: 27.828-86.258) por doenças cardiovasculares por ano. Com uma redução de 30% no consumo de sal, como recomendado pela Organização Mundial de Saúde até 2025, aproximadamente 1.188 mortes (95% II: 520-1.820), 2.100 casos incidentes (95% II: 923-3.234) e 27.272 DALYs (95% II: 11.999-41.675) por doenças cardiovasculares poderiam ser prevenidos ou adiados por ano.

Conclusão

Concluiu-se que a carga de doenças cardiovasculares atribuível ao consumo excessivo de sal no Paraguai é significante e políticas de redução deveriam ser priorizadas no país.

Palavras-chave: *Sal. Sódio. Doenças Cardiovasculares. Mortalidade. Hipertensão. Doenças Crônicas Não-Transmissíveis. Paraguai.*

INTRODUCTION

Non-communicable Diseases (NCDs) are a major cause of mortality globally. In the Americas they represent almost half of premature deaths. Projections for the future indicate that the burden of NCDs will continue to increase in low-income countries because of the aging of the populations, sedentarism and changes in diets [1,2]. The NCDs are related to four main risk factors: inadequate diets, abusive alcohol consumption, smoking and sedentarism and, in Paraguay, obesity is considered as an additional risk factor [3].

The NCDs are responsible for 41 million deaths per year, equivalent to 71% of global deaths. Every year, 15 million people from 30 to 69 years of age die from NCDs and over 85% of premature deaths occur in low and middle-income countries. Among NCDs, Cardiovascular Disease (CVD) is the major cause of death (17.9 million/year), followed by cancer (9.0 million), respiratory diseases (3.9 million) and diabetes (1.6 million). These four groups of diseases are responsible for over 80% of the premature deaths from NCDs [4].

The CVD are the main cause of morbimortality in Latin America and multifactorial risk factors, such as increased abdominal fat, hypertension, dyslipidemia, and intolerance to glucose, are associated with coronary heart disease, stroke, and heart failure. Therefore, the recent increase in CVD has become a scientific, health and socioeconomic priority, together with the need for innovation in interventions to prevent CVD through changes in lifestyle [5].

Hypertension is one of the main risk factors for death from CVD and the current body of evidence stresses the efficacy of reducing dietary salt to control hypertension and the incidence of CVD at the individual level. At the population level, lower average salt intake is associated with lower prevalence and mortality from CVD [6].

Excessive dietary salt consumption (more than 5 g of salt/day or 2,000 mg of sodium/day for a 2,000-kcal diet) is an important determinant of blood pressure in individuals and in populations.

Reducing dietary salt reduces blood pressure and, therefore, reduces the risk of CVD [7,8]. Because of this, the World Health Organization (WHO) considers salt reduction strategies as some of the most cost-effective interventions to reduce the global burden of NCDs [9].

Paraguay and other countries of the region face a double burden of malnutrition, which is represented by the coexistence of undernutrition with diet-related NCDs, and that has increased especially among the young population [10]. However, communicable diseases often associated with poverty remain relevant in the epidemiological scenario of the Paraguayan population and the COVID-19 pandemic has taken an important toll on the country. According to the Ministry of Public Health and Wellbeing, in the last 10 years, the prevalence of diabetes has increased from 6.5% to 9.7%, the prevalence of hypertension has increased from 35% to 45% and overweight has increased from 36% to 57% in the adult population. Meanwhile, regarding the underlying NCDs risk factors, only 16% of the population accomplish the requirement of fruit and vegetable consumption and the prevalence of physical inactivity and smoking are 25% and 14%, respectively. As a result, NCDs represent the four major causes of death during the last years in Paraguay and represented 50.1% of premature deaths in 2020 [11].

There are few available studies on the salt intake of the adult population of Paraguay. One of the only studies, through a 24-hour urine survey in health workers, has found a median equivalent to 13.7 g ± 5.4 g/day, which is over twice the recommendation of the World Health Organization [12].

Aiming to address this public issue, several national strategies have been implemented to excessive salt intake and generate better health for the population. The Dietary Guidelines of Paraguay state that "foods should be prepared using iodized salt in small quantities to prevent high blood pressure to incentivize the moderate consumption of iodized table salt because of the lack of other iodine sources in the diet of Paraguayans". Additionally, the National Resolution n° 792, of 2015, established maximum salt content of 600 mg of sodium per 100 grams of baked products, while local legislation determined that saltshakers must be withdrawn from restaurant tables [13].

Considering the current salt intake in Paraguay, this study aims to estimate the attributable deaths to excessive sodium consumption in the Paraguayan population in different scenarios and provide subsidies to public health surveillance and to decision-makers and stakeholders.

METHODS

This study provides an assessment of the health impact considering the reduction of deaths, incident cases and Disability Adjusted Life Years (DALYs) from cardiovascular diseases in the Paraguayan population in two scenarios of salt reduction using a comparative risk assessment model: (1) consumption of less than 5 g of salt per day, and (2) 30% reduction in the average salt consumption of Paraguayan adults (the national target for 2024 and the World Health Organization target for 2030).

This research complies with all ethical principles and informed consent was not necessary because the analysis was entirely based on public information from the government and international databases and open-access research, which are in the public domain, with no chance of identifying individual subjects [14,15]. This information will not be used outside the framework of this research.

Comparative Risk Assessment Analysis

We used the Preventable Risk Integrated Model (PRIME), which is a comparative risk assessment macrosimulation model for NCDs scenarios that associates risk factors, including diet

(and salt consumption) with health outcomes and estimates the impact of changes in the distribution of NCDs risk factors on adult population mortality, by age and sex groups. The PRIME model uses data from meta-analyzes of random controlled trials that associate salt consumption with hypertension and cardiovascular diseases. As inputs, the model required information on the population, deaths by cause and salt intake according to sex and age groups for the adult population. The PRIME model was developed by the University of Oxford and is available to users upon request [16].

The model compares the estimated impact of the risk factors to alternative (counterfactual) scenarios, in which the distribution of one or more risk factors is changed. For salt intake, the model assumes a log-linear distribution in the population using the average salt intake and its standard deviation from baseline and in the counterfactual scenarios and estimates the changes in the population attributable fraction for each age and sex group using the relative risks from meta-analysis, which is applied to the deaths by age and sex groups.

Within each sex and age stratum and for each scenario, we calculated the population attributable fraction at baseline and for each counterfactual scenario for six CVD subtypes (coronary heart disease, stroke, aortic aneurysm, hypertensive heart disease, rheumatic heart disease, and heart failure). Briefly, the population attributable fraction for outcome (o) in age group (a) and sex (s) was calculated as:

$$PAF_{oas} = \frac{\int_{x=0}^{m} RR_{oa}(\mathrm{x})\, P_{as}(\mathrm{x})dx - \int_{x=0}^{m} RR_{oa}(\mathrm{x})\, P'_{as}(\mathrm{x})dx}{\int_{x=0}^{m} RR_{oa}(\mathrm{x})\, P_{as}(\mathrm{x})dx}$$

Where Pas(x) and P'as(x) are the distributions of salt intake at the baseline and in the counterfactual scenario, respectively. RRoa(x) is the relative risk for cause-specific mortality (outcome 'o') as a function of salt consumption, according to the age group.

In parallel, we used the same parameters and rationale of the PRIME model to estimate the attributable CVD cases and disability adjusted life years (DALYs) considering the same counterfactual scenarios, as described by Trieu et al. [17].

Finally, the modeling incorporated the uncertainty of the epidemiologic inputs to the final outcomes using Monte Carlo simulations, with 10,000 iterations, which are expressed by the Uncertainty Intervals (UI) in the final estimates Model inputs.

The demographic and mortality data by age and sex were obtained from publicly available databases of the *Instituto Nacional de Estadística* (National Institute of Statistics of Paraguay) [13]. Additionally, we utilized estimates and uncertainties of the number of disease events (deaths, incident cases, and DALYs) in Paraguay during 2019 from the Global Burden of Disease study [15]. The deaths by age and sex group that were included in the model encompass coronary heart disease, stroke, hypertensive heart disease, heart failure, aortic aneurysm, and rheumatic heart disease (International Code of Disease – ICD 10: I20-25, I60-69, I10-15, I71, respectively).

Salt consumption baseline data was obtained from the research by Pedrozo et al. [12]. The counterfactual scenarios for modeling considered an average intake of 3.4 g ± 0.8 g of salt/day for men and women (population intake of less than 5 g/day, for both men and women) and of 10.4 g ± 2.5 g for men and 9.0 g ± 2.7 g for women (30% reductions in the average salt intake, according to sex) by the Paraguayan adult population. For the first counterfactual scenario, we modeled the population intake to fit a log-normal distribution with a maximum intake of 5 g/day to accommodate the WHO recommendation. In both scenarios, we assumed that the standard deviations remained at the same proportion of the mean as in the baseline scenario.

RESULTS

In 2019, CVD were responsible for 9,334 deaths, 16,671 incident cases of disease and 195,599 DALYs (Table 1) in Paraguay. The epidemiological burden of CVD in Paraguay is particularly significant in terms of total deaths (37%) and total DALYS (12%).

Table 1 – Estimated cardiovascular disease deaths, cases, DALYS and population in Paraguay, by sex and age group according to the Global Burden of Disease, 2019.

Men					Women					Total				
Age	Deaths	Cases	DALYs	Population	Age	Deaths	Cases	DALYs	Population	Age	Deaths	Cases	DALYs	Population
20-24	14	333	1,188	332,667	20-24	7	351	799	320,491	20-24	21	684	1,987	653,159
25-29	21	337	1,559	313,892	25-29	9	331	943	304,439	25-29	30	668	2,502	618,331
30-34	40	347	2,611	289,843	30-34	17	307	1,365	283,290	30-34	57	654	3,976	573,132
35-39	55	354	3,184	248,483	35-39	32	281	2,065	247,095	35-39	87	635	5,249	495,579
40-44	109	409	5,468	201,219	40-44	60	304	3,169	203,661	40-44	169	713	8,637	404,880
45-49	163	505	7,295	179,344	45-49	89	372	4150	180,716	45-49	252	877	7,710	360,060
50-54	269	729	10,572	158,119	50-54	144	493	5,846	158,707	50-54	413	1,222	16,418	316,826
55-59	405	1,008	13,887	135,739	55-59	205	629	7,206	135,072	55-59	610	1,637	21,093	270,811
60-64	523	1,174	15,414	114,889	60-64	274	730	8,264	112,397	60-64	797	1,904	23,678	227,286
65-69	621	122	15,491	86,873	65-69	349	785	8,839	86,776	65-69	970	907	24,330	173,649
70-74	650	101	13,366	57,895	70-74	422	735	8,743	60,540	70-74	1,072	836	22,109	118,435
75-79	684	799	11,186	37,988	75-79	540	701	8,849	43,033	75-79	1,224	1,500	20,035	81,021
>=80	1,597	1,091	15,641	38,098	>=80	2,034	1,337	18,499	55,813	>=80	3,631	2,428	34,140	93,911
Total	5,151	7,309	116,862	2,195,049	Total	4,182	7,356	78,737	2,192,032	Total	9,333	14,665	195,599	4,387,080

For the same year, according to our modeling analysis, it was estimated that approximately 2,656 deaths (95% UI, 1,250-3,765), corresponding to 28.5% of the total CVD deaths in Paraguay, could be prevented or postponed if the average per capita salt intake by the adult population was reduced to less than 5 g per day. Over half of these preventable deaths are premature (under 75 years of age) and almost 60% are among men (Table 2).

Table 2 – Cardiovascular disease events (deaths, incident cases and DALYs) averted from reducing population salt consumption to less than 5 g/day. Paraguay, 2019.

Metric and disease	Number of events averted (95% UI)	% CVD incidents averted
Deaths/year		
Cardiovascular diseases	2,656 (1,250-3,765)	28.5
Coronary heart disease	1,138 (521-1,652)	12.2
Stroke	944 (442-1,352)	10.1
Other	570 (284-774)	6.1
Men	1,573 (746-2,216)	30.5
Women	1,083 (503-1,549)	25.9
Under 75 years of age	1,463 (697-2,063)	15.7
Incident cases/year		
Cardiovascular diseases	4,816 (2,251-6,947)	28.9
Coronary heart disease	1,483 (683-2,154)	8.9
Stroke	2,524 (1,198-3,576)	15.1
Other	802 (333-1,280)	4.8
Men	2,883 (1,357-4,125)	30.9
Women	1,931 (891-2,821)	26.3
Under 75 years of age	3,930 (1,848-5,633)	23.6
DALYs/year		
Cardiovascular diseases	60,529 (27,828-86,258)	30.9
Coronary heart disease	26,067 (11,733-37,891)	13.3
Stroke	23,756 (10,899-33,877)	12.1
Other	10,579 (5,049-14,785)	5.4
Men	38,203 (17,733-54,047)	32.7
Women	22,230 (10,095-32,180)	28.3
Under 75 years of age	46,806 (21,687-66,506)	23.9

As also detailed in Table 2, in 2019, the major causes of prevented or postponed deaths attributable to excessive salt intake in the Paraguayan adult population were coronary heart disease and stroke, which together accounted for almost 80% of the total CVD deaths, followed by other CVD (hypertensive heart disease, heart failure, aortic aneurysm, and rheumatic heart disease). In addition, it was estimated that excessive salt intake among Paraguayan adults causes approximately 4,816 incident cases (95% UI, 2,251-6,947) and 60,529 DALYs (95% UI, 27,828 - to 86,258) from CVD yearly, with similar sex distribution as found for the attributable deaths to excessive salt intake. Compared to the deaths by age, however, both averted incident cases and DALYs from CVD for adults under 75 years of age represent a larger proportion of the total averted CVD incidents (77.5% and 82.0%, respectively), demonstrating even larger impacts of salt reduction compared to the averted deaths.

For 2019, if the WHO salt reduction targets (30% reduction in the average salt intake) were achieved, approximately 1,188 (95% UI, 520-1,820) deaths from CVD would be prevented or postponed, correspondent to 12.7% of the total deaths from CVD and 45% of the attributable deaths to excessive salt intake among Paraguayan adults. Again, deaths from coronary heart disease and stroke represent most of CVD deaths (76%) and deaths among people under 75 years of age represent 55% of the averted deaths (Table 3).

Table 3 – Cardiovascular disease events (deaths, incident cases and DALYs) averted if population salt consumption was reduced by 30%. Paraguay, 2019.

Metric and disease	Number of events averted (95% UI)	% CVD incidents averted
Deaths/year		
Cardiovascular diseases	1,188 (520-1,820)	12.7
Coronary heart disease	489 (213-757)	5.2
Stroke	418 (183-642)	4.5
Other	277 (122-421)	3.0
Men	695 (306-1,062)	13.5
Women	492 (214-758)	11.8
Under 75 years of age	657 (289-1,005)	7.0
Incident cases/year		
Cardiovascular diseases	2,100 (923-3,234)	12.6
Coronary heart disease	637 (279 to 981)	3.8
Stroke	1,128 (496-1,733)	6.8
Other	277 (122-421)	2.0
Men	1,237 (546-1,896)	13.3
Women	862 (377-1,338)	11.7
Under 75 years of age	1,721 (756-2,653)	10.3
DALYs/year		
Cardiovascular diseases	27,272 (11,999-41,675)	13.9
Coronary heart disease	11,364 (4,963-17,525)	5.8
Stroke	10,781 (4,761-16,477)	5.5
Other	5,113 (2,249-7,839)	2.6
Men	16,988 (7,502-25,870)	14.5
Women	10,285 (4,497-15,820)	13.1
Under 75 years of age	21,149 (9,344-32,119)	10.8

In this scenario of population salt reduction, approximately 2,100 incident cases (95% UI, 923-3,234) and 27,272 DALYs (95% UI, 11,999-41,675) from CVD would be averted. As for the previous scenario, averted incident cases and DALYs from CVD for people under 75 years of age would represent the majority (close to 80%) of the total averted CVD incidents.

DISCUSSION

The results of this study show that 28% of the deaths, 29% of incident cases and 31% of DALYs from cardiovascular disease could be prevented or postponed if the population's salt intake was reduced to 5 g/day, as recommended by the WHO. Over half of these preventable deaths would be premature and they represent a significant epidemiological burden to Paraguay. In 2019, the mortality rate from CVD was 161,8 (95% UI 107.4-232.2) per 100,000 habitants and ranks this country in quintile 3 (40-60%) among all countries in the region of the Americas (22). Additionally, if Paraguay achieved the WHO salt reduction goals for 2025 (30% reduction in the population intake), the prevented or postponed deaths, incident cases and DALYs from CVD would also be significant (13% to 14% reductions).

This study is the first comparative risk assessment model performed in Paraguay and its results are compatible with those of modeling studies in Brazil [18], Costa Rica [19] and Argentina [20]), which stress the burden of excessive salt intake on morbimortality in Latin American countries and represents important evidence for policymaking and for prioritizing salt reduction in Paraguay.

In response to the global health issue of excessive salt intake, WHO has urged countries to achieve a 30% relative reduction in average population salt intake by 2025 and developed the SHAKE technical package, with a set of tools, resources and real case examples that can guide the design, implementation, monitoring and evaluation of national salt reduction programs and provides a general framework for the key elements needed to formulate an effective strategy to reduce salt consumption. The key areas for action include political commitment, programmatic leadership, raising awareness and building partnerships divided into sectors such as population salt consumption surveillance, civil society mobilization, adoption of standards, knowledge or education and healthy food environments [21].

There are several dietary sources of salt in the populations and, in Latin America, commonly table salt and salt-based condiments represent an important part of salt intake [22]. However, the participation of processed and ultra-processed foods has significantly increased, so multicomponent policies are needed to achieve the reduction of salt intake by the population, and may include food reformulation, communication and education, food procurement policies, food labelling, taxation over unhealthy food, regulation of food marketing and implementation of food-based dietary guidelines [22,23].

According to a recent mapping of the implementation of salt reduction strategies by Pan American Health Organization (PAHO), in Latin American countries, while Brazil and Paraguay have implemented two "best buy" (reformulation of food products and establishment of a supportive environment in public institutions), Argentina and Uruguay 2021 have completed three "best buy" strategies, including front-of-pack nutritional labelling. On the other hand, Bolivia, another neighbor country of Paraguay, has not yet implemented any "best buy" strategy [13].

Nevertheless, in Paraguay, the comprehensive political framework required to implement the SHAKE package is still under construction, because of the lack of human, economic and logistical resources hinders the monitoring and modification of food environments. Also, the country requires more active citizen participation in health and nutrition to promote civil advocacy for effective policies and to support population empowerment and educational campaigns. Nevertheless, the results of this study may help support the national prioritization of this issue.

Because of the increased participation of industrialized foods in most countries and the high salt content in many food categories, food reformulation has been a key strategy among salt

reduction policies. Following the example of the United Kingdom, where voluntary salt reduction targets were set between the government and food industries and achieved important reductions in salt intake which impacts on health [24], several countries in the Americas, including Brazil, Canada, Costa Rica and the United States have adapted this strategy through maximum limits for salt content in relevant food categories that were voluntarily agreed on by food industries. In parallel, after starting with voluntary targets in Argentina, mandatory limits for salt reduction were implemented in the country [13].

Although the voluntary salt reduction targets have projected impacts on salt intake and health outcomes, regulatory targets have proven to be more effective, because they reach all products in the food market and can be strongly enforced by health authorities [25]. For example, in Brazil, although the voluntary targets have reduced salt intake in foods and salt intake in the population [26], if mandatory targets were implemented in the country, adopting lower international benchmarks, the reduction of salt intake could be more than three times larger [27].

In this field, the PAHO has proposed the first regional targets for salt reduction in the Americas, based on the national targets in the region, in 2015, which have been recently expanded and updated from 18 categories to 16 categories with 75 subcategories [28]. Additionally, the WHO has also set global benchmarks for the sodium content for 18 food categories and 97 subcategories to support countries in their salt reduction policies [29].

According to a recent regional multicentric project on salt reduction policies in Latin America, in Paraguay, 87.9% of packaged food products met the PAHO regional targets for 2015 and bouillon cubes and powders presented the highest sodium content among food categories [29].

It is also important to consider the participation of table salt in diets, as well as other foods in salt reduction policies. For example, in 2018-2019, the street, artisanal and fast foods from Paraguay and Brazil had the highest salt content compared to Argentina, Costa Rica and Peru. Additionally, countries including Paraguay have developed the framework for a social marketing strategy aimed at female caregivers of school-aged children, considering the benefits and barriers of reducing the use of table salt and salt-based condiments and replacing them with natural and less processed ingredients with less or no salt [30,31].

This is the first modeling study on salt reduction in Paraguay, which is an important milestone for advocacy in the country and it represents a baseline for future studies that may analyze specific policy scenarios for salt reduction and other NCDs policies, as well as including health economic outcomes, such as the direct and indirect costs of disease and dietary risk factors.

Some of the limitations of this study are related to data inputs. Although demographic and mortality data are nationally representative of Paraguay, the only existing estimates of salt intake do not allow disaggregation by age and were obtained from a 24-hour urine study in Asunción, the country's capital, in 2014. Therefore, the analysis is based on the assumption that the national salt intake is equivalent to that of the population of the capital and that it has remained stable from 2014 to 2019. In addition, part of the baseline data was based on the Global Burden of Disease study and shares the strengths and limitations of its methodology.

Comparative risk assessment models are relatively simple macrosimulation tools for estimating the impact of changes in the population health when exposed to risk factors, such as salt intake through a cross-sectional analysis. This type of modeling is based on high-quality meta-analyzes and has been validated and used in several countries [16-18]. However, the inherent limitations of these models include the absence of a time variable for the time lag between changes in risk factors and the impact on health outcomes and does not incorporate the assessment of recurring events and the possible interactions between individuals and populations.

CONCLUSION

Considering the deaths, incident cases and DALYs from CVD in Paraguay, approximately 30% of the total burden of CVD would be prevented or postponed if the Paraguayan population's salt intake was reduced to less than 5 g/day and significant health benefits would be achieved through intermediate reductions. These results demonstrate the need for prioritizing salt/sodium reduction policies in the general population of Paraguay.

Indeed, this kind of simulation study is very helpful to the national health authorities together with other government areas, industry, health professionals and the general population, for increasing awareness of the burden of the NCDs and specific risk factors, such as excessive salt intake, and may help designing and evaluating effective policy options for preventing death and morbidity in the country.

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CONTRIBUTORS

EAF NILSON, E SANTACRUZ and R BURGOS conceived and contributed to the design of the study. R BURGOS contributed to the acquisition of data for the work. EAF NILSON contributed to the analysis and interpretation of data for the work. EAF NILSON and R BURGOS drafted the manuscript. R BURGOS, D DUARTE-ZOILAN, C TURNES, G BENITEZ, E SANTACRUZ and E EAF NILSON revised and approved the final the manuscript.