



2017; v. 7; n. 4

Original Article

Food habits and sociodemographic profile of patients with pulmonary tuberculosis

Hábito alimentar e perfil sociodemográfico de pacientes com tuberculose pulmonar

Hábito alimentar y perfil sociodemográfico de pacientes con tuberculosis pulmonar

Andréa das Graças Ferreira Frazão¹, Rosa Maria Dias¹, Jessica Cordeiro dos Santos Sousa¹, Carlos Augusto Abreu Alberio¹, José Luis Fernandes Vieira¹, Givago da Silva Souza¹

¹Universidade Federal do Pará, Belém, PA, Brazil

Submitted on: 07/27/2017

Accepted on: 09/14/2017

deabrelaz@gmail.com

ABSTRACT

Background and Objectives: Tuberculosis (TB) is an infectious disease and people with nutritional deficiencies may be more susceptible to it. The aim of this study was to investigate dietary markers and their association with sociodemographic variables of patients with pulmonary TB. **Methods:** A cross-sectional descriptive study carried out between 2015 and 2016 in Belém-PA. TB patients were interviewed using a questionnaire that was developed based on the National Health Survey, including questions about sociodemographic variables and healthy food markers. Data were analyzed in the Epi-Info and Bioestat programs, using the G and Binomial tests, with a significance level of 5%. **Results:** 61 patients participated in the study, most of them males (62.2%), aged 40-59 years old (42.6%) and educational level with 10 to 12 years of schooling (42.6%). There was a statistically significant association for the consumption of the three markers ($p < 0.00$), as well as between gender and recommended fish consumption ($p = 0.00$). **Conclusion:** The food habits of healthy food markers below the recommended frequency can modify the nutritional profile of pulmonary TB patients and to facilitate infections and the consequences of the disease.

KEYWORDS: Healthy Diet. Tuberculosis, Pulmonary. Disease Prevention.

RESUMO

Justificativa e Objetivos: A tuberculose (TB) é uma doença infecciosa que pode ser mais suscetível em pessoas com carências nutricionais. O objetivo deste estudo foi investigar

marcadores alimentares e sua associação com variáveis sociodemográficas dos pacientes com TB pulmonar. **Métodos:** Estudo descritivo do tipo transversal realizado entre 2015 e 2016, em Belém-PA. Foram entrevistados pacientes com TB por meio de um questionário elaborado com base na Pesquisa Nacional de Saúde, com questões sobre variáveis sociodemográficas e marcadores de alimentação saudável. Os dados foram analisados nos programas Epi-Info e Bioestat, a partir dos testes G e Binomial, com nível de significância de 5%. **Resultados:** Participaram 61 pacientes, a maioria era do sexo masculino (62,2%), faixa etária entre 40-59 anos (42,6%) e nível de escolaridade de 10 a 12 anos de estudo (42,6%). Houve associação estatisticamente significativa para o consumo dos três marcadores ($p < 0,00$), bem como entre o sexo e o consumo recomendado de peixe ($p = 0,00$). **Conclusão:** O hábito alimentar de marcadores de alimentação saudável abaixo da frequência recomendada pode modificar o perfil nutricional do paciente de TB pulmonar e facilitar a infecção e as consequências da doença.

DESCRITORES: Dieta Saudável. Tuberculose Pulmonar. Prevenção de Doenças.

RESUMEN

Justificación y Objetivos: La tuberculosis (TB) es una enfermedad infecciosa que puede ser más susceptible en personas con carencias nutricionales. El objetivo del estudio fue investigar marcadores alimentarios y su asociación con variables sociodemográficas de pacientes con TB pulmonar. **Métodos:** Estudio descriptivo, del tipo transversal realizado entre 2015 y 2016, en Belém-PA. Fueron entrevistados pacientes con TB a través de un cuestionario elaborado con base en la Encuesta Nacional de Salud, con cuestiones sobre variables sociodemográficas y marcadores de alimentación saludable. Los datos fueron analizados en los programas Epi-Info y Bioestat, a partir de las pruebas G y Binomial, con un nivel de significancia del 5%. **Resultados:** Participaron 61 pacientes, la mayoría era del sexo masculino (62,2%), rango etario entre 40-59 años (42,6%) y nivel educativo de 10 a 12 años de estudio (42,6%). Ha habido una asociación estadísticamente significativa para el consumo de los tres marcadores ($p < 0,00$), así como entre el sexo y el consumo recomendado de pescado ($p = 0,00$). **Conclusiones:** El hábito alimentario de marcadores de alimentación saludables por debajo de la frecuencia recomendada puede modificar el perfil nutricional del paciente de TB pulmonar y facilitar la infección y las consecuencias de la enfermedad.

PALABRAS CLAVE: Dieta Saludable. Tuberculosis Pulmonar. Prevención de Enfermedades.

INTRODUCTION

Tuberculosis (TB) is an infectious disease and individuals with nutritional deficiencies can be more susceptible to its occurrence. The usual consumption of food, sources of nutrients, is a factor involved in susceptibility to infection, but remains scarcely studied in TB.¹

TB is caused by *Mycobacterium tuberculosis*, which affects mainly the lungs, and is the leading cause of death by an infectious agent worldwide, more than the deaths caused by AIDS and malaria together. There were an estimated 10.4 million new cases of TB and 1.4 million deaths from the disease in 2015 worldwide.²

In the same year, Brazil remained among the 30 countries with the highest disease burden.² The North region had the highest incidence rates (41.8/100,000 inhabitants) of the disease in the country. Among the capital cities, Belém was the third with the highest risk of death (5.1/100,000 inhabitants) from TB in 2015.³

The association between pulmonary TB and nutrition has been pointed out as an important aspect for the effectiveness of the host's immune response to the disease, since protein catabolism, cytokine activation and modifications in the immune system and gastrointestinal tract may occur, resulting in malnutrition and / or nutritional deficiencies.^{4,5}

Malnutrition constitutes an important indicator of the risk of contracting TB and may also influence treatment efficacy, considering that the administration of standardized drugs can cause adverse effects, including liver and gastrointestinal alterations.^{6,7} These alterations may result in modifications of the usual diet of patients with pulmonary TB, aiming to improve drug absorption and increase the supply of specific nutrients, such as proteins, vitamins and minerals.^{8,9}

Adopting eating habits depends on access to food, economic, political, cultural and social conditions.¹⁰ Therefore, the sociodemographic profile of individuals with TB indicates that it is a disease associated with poverty, and that in addition to early diagnosis, adequate treatment and epidemiological surveillance, the reduction of social inequalities and adequacy of control programs, such as knowledge on the eating habits and the local realities of the populations, are considered crucial aspects in the reduction of TB morbimortality.¹¹

In Brazil, the Ministry of Health recommends to the overall population, the preferential consumption of foods such as fruits and vegetables, beans and fish, among others.¹⁰ These foods have been considered markers of a healthy dietary pattern, as well as an indicator that the individual is ingesting the nutrients required for health maintenance.¹²

Due to the significant contribution of food and its nutrients to the recovery process of patients with pulmonary TB, this study aimed to investigate dietary markers and their association with sociodemographic variables of patients with pulmonary TB.

METHODS

This is a descriptive cross-sectional study, carried out from January 2015 to August 2016 at Centro de Saúde Escola do Marco of Universidade do Estado do Pará, in

the municipality of Belém-PA. Centro de Saúde Escola do Marco is a teaching and care unit located in a central district of the municipality and has a qualified multiprofessional team, constituting a reference unit in the care of patients with pulmonary TB.

The study population consisted of all adult and elderly patients of both genders diagnosed with pulmonary TB. The inclusion criteria comprised patients aged 18 years or over, with a bacilloscopic, radiological and clinical diagnosis of pulmonary TB undergoing treatment for the disease, in the abovementioned period and health unit.

The patients who met the inclusion criteria were characterized as primary infection, recurrence and / or re-admission after treatment abandonment.

Patients with multidrug-resistant TB, TB / HIV coinfection, diabetic, hypertensive, and pregnant patients, as well as patients with renal or hepatic impairment were excluded from the study, considering that these conditions interfere with eating habits due to dietary restrictions, modifications and inclusions.

The eating habits of the interviewed patients were investigated through a questionnaire, which was created based on the National Health Survey, after a pilot testing with 10 pulmonary TB patients, at the end of their treatment at Centro de Saúde do Marco.¹² It should be noted that the interviews were performed by two trained nutritionists who were not part of the care team of the selected unit.

This study considered as healthy food markers the recommended consumption of beans on five days of the week; recommended consumption of fruits and natural fruit juice and vegetables, including the consumption of green vegetables, comprising at least one serving of fruits or fruit juice and two servings of vegetables five times a day and five times a week, and the recommended fish consumption at least one day a week. These markers and their daily and weekly amounts were established by the National Health Survey for the Brazilian population.¹²

Moreover, the following variables were considered to characterize the patients' sociodemographic profile: gender, age group and educational level in years of schooling.

Descriptive statistics were used for the data analysis, using the Epi Info program and inferential statistics to verify associations between variables related to healthy food markers and sociodemographic variables. As a complementary analysis, the non-parametric tests for the two independent populations (G Test) and the Binomial test were used to analyze the difference between two independent proportions in the Bioestat program. The level of significance was set at 5%.

The patients who agreed to participate in this study signed the free and informed consent form, which was approved by the Research Ethics Committee of the Tropical Medicine Division of Universidade Federal do Pará (UFPA), according to Opinion n.865.243 / 2014, in compliance with National Health Council Resolution n. 466/2012.¹³

RESULTS

Of the 88 TB patients diagnosed in Centro de Saúde Escola do Marco, 69 (78.4%) had the pulmonary clinical form. Of these, eight were excluded, seven of them due to multidrug-resistant pulmonary TB and one was coinfecting with TB/HIV. Thus, 61 patients with pulmonary TB were selected for the study.

Most patients were males (62.3%), aged between 40 and 59 years (42.6%), followed by 25 to 39 years (36.1%), with an educational level of 10 to 12 years of schooling (42.6%) (Table 1).

Table1 – Distribution of the sociodemographic profile of pulmonary TB patients interviewed for the study, according to gender in Belém, Pará, 2015-2016.

Sociodemographic variables	Gender				Total	
	Female		Male		N	%
	N	%	N	%		
Age range						
18 to 24	3	4.9	7	11.5	10	16.4
25 to 39	9	14.8	13	21.3	22	36.1
40 to 59	9	14.8	17	27.8	26	42.6
≥ 60	2	3.3	1	1.6	3	4.9
Total	23	37.8	38	62.2	61	100
Years of schooling						
0 to 4	7	11.5	12	19.7	19	31.2
5 to 9	2	3.3	4	6.5	6	9.8
10 to 12	9	14.7	17	27.9	26	42.6
>12	5	8.2	5	8.2	10	16.4
Total	23	37.7	38	62.3	61	100

It was observed that the consumption of vegetables and fruits was below the recommended allowance (54.1%). Moreover, the consumption of the three healthy food markers below the recommended allowance was reported by 82.0% of the patients, with a statistically significant difference ($p < 0.00$) (Table 2).

Table 2 – Frequency of consumption of healthy food markers by pulmonary TB patients interviewed in Belém, Pará, 2015-2016.

Frequency of consumption	Yes		No		Total		p*
	N	%	N	%	N	%	
Recommended Consumption of Beans ¹	35	57.4	26	42.6	61	100	0.43
Recommended consumption of vegetables and fruits ^{1,2}	28	45.9	33	54.1	61	100	0.71
Recommended Consumption of fish ³	39	63.9	22	36.1	61	100	0.10
Recommended Consumption of the three markers ^{1,2,3}	11	18.0	50	82.0	61	100	<0.00

* G Test. ¹ Consumption on five or more days of the week; ²Consumption five or more times a day; ³Consumption on at least one day of the week.

The proportion of men who consumed fish was statistically significant ($p = 0.00$) when compared to the proportion of women. Regarding the age group and educational levels in years of schooling, there were no statistically significant differences regarding the consumption of healthy food markers (Table 3).

Table 3 – Distribution of the sociodemographic profile of patients with pulmonary TB interviewed for the study according to healthy food markers in Belém, Pará, 2015-2016.

Sociodemographic variables	Healthy food markers					
	Recommended Consumption of beans ¹ n (%)	p	Recommended Consumption of fruits and vegetables ^{1,2} n (%)	p	Recommended Consumption of fish ³ n (%)	p
Gender						
Male	20 (52.6)	0.33 [‡]	15 (39.4)	0.19 [‡]	31 (81.5)	0.00 [‡]
Female	15 (65.2)		13 (56.5)		08 (34.7)	
Age range (years)						
18-24	07 (70.0)	0.89*	03 (30.0)	0.87*	06 (60.0)	0.98*
25-39	14 (63.6)		10 (45.4)		13 (59.0)	
40-59	13 (50.0)		14 (53.8)		18 (69.2)	
≥ 60	01 (33.3)		01 (33.3)		02 (66.6)	
Educational level in years of schooling						
0-4	10 (52.6)	0.87*	04 (21.0)	0.30*	19 (100.0)	0.92*
5-9	02 (33.3)		03 (50.0)		04 (66.6)	
10-12	17 (65.3)		13 (50.0)		14 (53.8)	
≥ 12	06 (60.0)		08 (80.0)		07 (70.0)	

[‡] Binomial test; *G test; ¹ Consumption on five or more days of the week; ²Consumption five or more times a day; ³Consumption on at least one day of the week.

DISCUSSION

The results of this study showed a statistically significant association between the simultaneous consumption, below the recommended frequency, of the three healthy food markers ($p < 0.00$). This finding suggests that the eating habits of patients with pulmonary TB possibly showed nutritional deficiencies, which may have implications in the treatment process and health status recovery.⁴

Regarding the sociodemographic variables, the results show a predominance of pulmonary TB in male patients (62.2%), in agreement with other studies, considering the greater exposure to risk factors, lower demand for health services and cultural habits related to male behavior in situations of illness.^{2,11,14}

The age range of the patients in the present study was young, ranging from 40 to 59 years old (42.6%), and comprised the economically active population, usually responsible for the care of younger and older individuals, which may include a greater number of people affected by the disease and its consequences, such as eating habits.¹⁵

The educational level of 10 to 12 years of schooling, reported by 42.6% of the interviewed patients, implies an improvement in self-care, as it would allow greater access to information, knowledge on TB and the importance of food habits. However, this finding differs from other studies that indicated a lower educational level in patients with pulmonary TB.^{14,15}

Among the healthy food markers, 42.6% of the respondents' consumption of beans was below the recommended level, which allows the deficiency of vegetable protein, among other nutrients.¹⁰ Protein plays an important role in T cell-mediated immune function, by increasing the resistance to infectious diseases, such as TB.¹⁶

Regarding the consumption of fruits and vegetables, 54.1% of those interviewed did not consume these foods at the recommended amount, which could contribute to the reduction of the antioxidant action in the cells, in addition to hindering the inflammatory processes inhibition that occurs in infectious diseases.¹⁷

In this study 36.1% of the interviewed patients did not consume the amount of fish as recommended by healthy consumption guidelines, considered a protective food due to the large amount of omega-3 polyunsaturated fatty acids. These substances have anti-inflammatory properties and may contribute to limit inflammatory processes observed at the beginning of the TB infection.¹⁸

A statistically significant association was observed between gender and the recommended fish consumption, which was higher in men. This result is similar to the

findings of the National Health Survey, mainly in the states of the North Region due to its availability of fish in relation to other regions.^{12,19} However, no scientific evidence was found to explain the higher consumption of fish among men when compared to the women. A possible explanation for this finding may be the place of residence of some male patients, who reported residing on the islands near Belém-PA, thus having greater food availability, both through purchasing and direct acquisition by fishing in rivers.

The absence of a statistically significant association of the recommended consumption of beans, vegetables and fruits with gender, found in this study, is similar to the results found in a study carried out with the population assisted by a nutritional education program in Goiânia (state of Goiás, Brazil).²⁰ On the other hand, it differs from the results found in a study carried out with adult and elderly patients treated at health units in Belo Horizonte, state of Minas Gerais, Brazil, which found a statistically significant association between inadequate consumption of fruits and vegetables and gender.²¹

No statistically significant association was observed between the recommended consumption of the three healthy food markers and the age group of the interviewed patients. It is noteworthy that of the three elderly subjects in the study group, none simultaneously consumed the three healthy food markers analyzed. This finding is a matter of concern, since the population aging process associated with an increase in the frequency of excess weight, sedentary lifestyle and changes in dietary patterns, such as the increase in the consumption of sugars and soft drinks instead of fruits, vegetables and green vegetables, have been pointed out as possible factors involved in the increased occurrence of diabetes mellitus and TB control.²²

It was not possible to verify the association between the level of schooling and the recommended consumption of beans, fish, fruits and vegetables. The importance of schooling for the understanding of pulmonary TB consequences and treatment adherence, including the adoption of food habits, such as the recommended consumption of the three markers addressed in this study is emphasized, as well as the effect of anti-TB drugs and health promotion.²³

The consumption of healthy foods should be stimulated since the diagnosis of pulmonary TB, as both malnutrition and excess weight interfere with immunological functions, increasing susceptibility to infections.²⁴ The nutritional status, which depends, among other factors, on the food consumed, is an important condition for the immune response modulation regarding the prognosis of infectious diseases.²⁵

Epidemiological studies such as the present one neither prove, nor exclude causality. To obtain causality it is necessary that other studies explore the association between pulmonary TB, eating habits and sociodemographic aspects, within the same research scenario. The validity of this study comes from its epidemiological design and the description of evidence on the association between sociodemographic factors and food habits of patients with pulmonary TB.

The study results showed that most pulmonary TB patients that were interviewed did not simultaneously consume the three healthy food markers at the recommended frequency. Among the sociodemographic variables, it was observed that only male gender was associated with higher fish consumption. Therefore, the habit of consuming these healthy food markers below the recommended frequency can modify the nutritional profile of pulmonary TB patients and facilitate the onset of infections and the consequences of the disease.

REFERENCES

1. Frediani JK, Sanikidze E, Kipiani M, et al. Macronutrient intake and body composition changes during anti-tuberculosis therapy in adults. *Clin Nutr* 2016; 35 (1): 205-212. <http://dx.doi.org/10.1016/j.clnu.2015.02.007>
2. World Health Organization (WHO). Global tuberculosis report 2016 [Internet]. Geneva: WHO, 2016 [citado 2017 jul 13]. 214 p. Disponível em: http://who.int/tb/publications/global_report
3. Ministério da Saúde (BR). Secretaria de Vigilância em Saúde. Boletim Epidemiológico. Indicadores prioritários para o monitoramento do Plano Nacional pelo fim da Tuberculose como problema de saúde pública no Brasil [Internet]. Brasília: Ministério da Saúde; 2017 [citado 2017 set 1]; 48 (8): 1-11. Disponível em: <http://portalarquivos.saude.gov.br/images/pdf/2017/marco/23/2017-V-48-N-8-Indicadores-priorit-rios-para-o-monitoramento-do-Plano-Nacional-pelo-Fim-da-Tuberculose-como-Problema-de-Sa--de-P--blica-no-Brasil.pdf>
4. Miyata S, Tanaka M, Ihaku D. The prognostic significance of nutritional status using malnutrition universal screening tool in patients with pulmonary tuberculosis. *Nutr J* 2013; 12 (42): 1-5. <http://dx.doi.org/10.1186/1475-2891-12-42>
5. Francisqueti FV, Pereira PCM. Terapia nutricional em paciente com tuberculose e gastrectomia: relato de caso. *Rev Bras Nutr Clin* 2012; 21 (1): 65-68.

6. Cegielski JP, Arab L, Cornoni-Huntley J. Nutritional risk factors for tuberculosis among adults in the United States, 1971–1992. *Am J Epidemiol* 2012; 176: 409–422. <https://doi.org/10.1093/aje/kws007>
7. Magis-escurra C, Van den Boogaard J, Ijdema D, et al. Therapeutic drug monitoring in the treatment of tuberculosis patients. *Pulm Pharmacol Ther* 2012; 25 (1): 83-86. <http://dx.doi.org/10.1016/j.pupt.2011.12.001>
8. Arbex MA, Varella MCL, Siqueira HR, et al. Drogas antituberculose: interações medicamentosas, efeitos adversos e utilização em situações especiais – parte 1: fármacos de primeira linha. *J Bras Pneumol* 2010; 36 (5): 626-640. <http://dx.doi.org/10.1590/S1806-37132010000500016>
9. Hall RG, Swancutt MA, Meek C, et al. Ethambutol pharmacokinetic variability is linked to mass in overweight, obese, and extremely obese people. *Antimicrob Agents Chemother* 2012; 56 (3): 1502-1507. <http://dx.doi.org/10.1128/AAC.05623-11>
10. Ministério da Saúde (BR). Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Guia alimentar para a população brasileira. Brasília: Ministério da Saúde; 2014. 156 p.
11. San Pedro A, Oliveira RM. Tuberculose e indicadores socioeconômicos: revisão sistemática da literatura. *Rev Panam Salud Publica* 2013; 33 (4): 294-301. <http://dx.doi.org/10.1590/S1020-49892013000400009>
12. Instituto Brasileiro de Geografia e Estatística. Pesquisa Nacional de Saúde 2013: percepção do estado de saúde, estilos de vida e doenças crônicas. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística; 2014.
13. Conselho Nacional de Saúde (BR). Resolução nº 466, 2012. Diretrizes e Normas regulamentadoras de pesquisa envolvendo seres humanos. Brasília, 13 jun. 2013. Seção 1 p. 59.
14. Alves RH, Reis DC, Viegas AM, et al. Epidemiologia da tuberculose no município de Contagem, Minas Gerais, Brasil, entre 2002 e 2011. *Rev Epidemiol Control Infect* 2014; 4 (2): 146-153. <http://dx.doi.org/10.17058/reci.v4i2.4411>
15. Freitas WMTM, Santos CC, Silva MM, et al. Perfil clínico-epidemiológico de pacientes portadores de tuberculose atendidos em uma unidade municipal de saúde de Belém, Estado do Pará, Brasil. *Rev Pan-Amaz Saude* 2016; 7 (2): 45-50. <http://dx.doi.org/10.5123/S2176-62232016000200005>
16. Lapa e Silva JR. Novos aspectos da patogenia da tuberculose. *Pulmão RJ* 2012; 21 (1):10-14.

17. Wynn E, Krieg MA, Lanham-New SA, et al. Postgraduate Symposium Positive influence of nutritional alkalinity on bone health. *Proc Nutr Soc* 2010; 69 (1): 166-173. <http://dx.doi.org/10.1017/S002966510999173X>
18. Siriwardhana N, Kalupahana NS, Moustaid-Moussa N. Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. *Adv Food Nutr Res* 2012; 65: 211-222. <https://doi.org/10.1016/B978-0-12-416003-3.00013-5>
19. Instituto Brasileiro de Geografia e Estatística - IBGE. Pesquisa de orçamentos familiares 2008-2009: análise do consumo alimentar pessoal no Brasil/IBGE, Coordenação de Trabalho e Rendimento. Rio de Janeiro: IBGE; 2011.
20. Silva MS, Silva NB, Alves AGP, et al. Risco de doenças crônicas não transmissíveis na população atendida em Programa de Educação Nutricional em Goiânia (GO), Brasil. *Cien Saude Colet* 2014; 19 (5): 1409-1418. <http://dx.doi.org/10.1590/1413-81232014195.16312013>
21. Oliveira MS, Lacerda LNL, Santos LC, et al. Consumo de frutas e hortaliças e as condições de saúde de homens e mulheres atendidos na atenção primária à saúde. *Cienc Saude Colet* 2015; 20 (8): 2313-2322. <http://dx.doi.org/10.1590/1413-81232015208.18272014>
22. Sá NNB, Moura EC. Excesso de peso: determinantes sociodemográficos e comportamentais em adultos, Brasil, 2008. *Cad Saude Publica* 2011; 27 (7): 1380-1392. <http://dx.doi.org/10.1590/S0102-311X2011000700013>
23. Belchior AS, Arcêncio RA, Mainbourg EMT. Differences in the clinical-epidemiological profile between new cases of tuberculosis and retreatment cases after default. *Rev Esc Enferm USP* 2016; 50 (4): 619-625. <http://dx.doi.org/10.1590/S0080-623420160000500012>
24. Krawinkel MB. Interaction of Nutrition and Infections Globally: an Overview. *Ann Nutr Metab* 2012; 61 (Suppl 1): 39-45. <http://dx.doi.org/doi:10.1159/000345162>
25. Chang SW, Pan WS, Beltran DL, et al. Gut Hormones, Appetite Suppression and Cachexia in Patients with Pulmonary TB. *PLoS One* 2013; 8 (1): e54564. <https://doi.org/10.1371/journal.pone.0054564>