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78 CASES OF METHEMOGLOBINEMIA INDUCED BY VEGETABLE INTAKE IN INFANTS IN NORTH SPAIN. A CASE-CONTROL STUDY

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Abstract:	Acquired methemoglobinemia (MHb) induced in infants by intake of vegetables is a condition uncommonly reported in the literature. The purpose of this study was to study new vegetables involved and other epidemiological risk factors. METHODS Seventy eight cases of diet-induced MHb seen in Pamplona from 1987 to 2010 are reported. Infant characteristics were collected, and a case-control study was conducted using as controls 78 age- and sex-matched infants selected at the same geographic area. Bivariate logistic regression analyses were performed to detect factors involved in MHb occurrence. SPSS 17 statistical software was used. Nitrate levels were tested in natural vegetables used to prepare purées. RESULTS A clear relationship was found between MHb and use of borage (Borago officinalis) (O =5.2; 95%CI=1.1-24.6) and maybe chard (Beta vulgaris var. cicla) (OR=2.0; 95%CI=0.4-8.7), time from preparation to use (OR=17.4, 95%CI=3.5-86.3 if the purée had been prepared 24-48 hours before and OR=24.9, 95%CI=3.3-187.6 if prepared more than 48 hours before), and breastfeeding (OR=10.4; 95%CI=1.9-57.2). Tests

	confirmed that vegetables with the highest nitrate levels were borage (n = 15), with mean nitrate levels of 3968 mg/kg, and chard (n = 17), with mean levels of 2811 mg/kg. CONCLUSIONS The main associated factors were shown to be time from purée preparation to use (> 24 hours), use of certain vegetables (borage and chard), and breastfeeding. Nitrate levels in both vegetables implicated as etiological factors in acquired MHb are very high.

Dear reviewers,

After analyzing your corrections and suggestions, all have seemed wise and timely, so we have decided to modify the manuscript to respond to each and every one of the corrections in the best way possible.

We remain at your disposal for any clarification or any other suggestion that you look that can help to improve the quality of the paper

Yours sincerely

Francisco Gil

Dear editorial board of Journal of Pediatric Gastroenterlogy and nutrition,
Please find enclosed the manuscript: "78 cases of methemoglobinemia induced by vegetable intake in infants in north spain. A case-control study", by Alba Martinez, et al., to be submitted as an Original article to the Journal of Pediatric Gastroenterlogy and nutrition for consideration of publication.
All co-authors have seen and agree with the contents of the manuscript and there are no conflict interests to report. We give witness that the study is set to the ethical standards appropriate to this type of work. We certify that the submission is original work and has not been published and is not currently under review at any other publication.
It has been hypothesized that diet-induced MHb in infants may be due to intake of nitrate-rich vegetables. Our study showed implication in MHb of borage and chard, time from preparation to use of home-made purée, storage, and breastfeeding.
We hope that the editorial board will agree on the interest of this study.
Attached are the names of three possible reviewers:

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Sincerely yours,
Francisco Gil on behalf of the authors

78 CASES OF METHEMOGLOBINEMIA INDUCED BY VEGETABLE INTAKE IN INFANTS IN NORTH SPAIN. A CASE-CONTROL STUDY

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ABSTRACT

Acquired methemoglobinemia (MHb) induced in infants by intake of vegetables is a condition uncommonly reported in the literature. The purpose of this study was to study new vegetables involved and other epidemiological risk factors.

Methods

Seventy eight cases of diet-induced MHb seen in Pamplona from 1987 to 2010 are reported. Infant characteristics were collected, and a case-control study was conducted using as controls 78 age- and sex-matched infants selected at the same geographic area. Bivariate logistic regression analyses were performed to detect factors involved in MHb occurrence. SPSS 17 statistical software was used. Nitrate levels were tested in natural vegetables used to prepare purées.

Results

A clear relationship was found between MHb and use of borage *(Borago officinalis*) (O =5.2; 95%CI=1.1-24.6) and maybe chard *(Beta vulgaris var. cicla)* (OR=2.0; 95%CI=0.4-8.7), time from preparation to use (OR=17.4, 95%CI=3.5-86.3 if the purée had been prepared 24-48 hours before and OR=24.9, 95%CI=3.3-187.6 if prepared more than 48 hours before), and breastfeeding (OR=10.4; 95%CI=1.9-57.2). Tests confirmed that vegetables with the highest nitrate levels were borage (n = 15), with mean nitrate levels of 3968 mg/kg, and chard (n = 17), with mean levels of 2811 mg/kg.

Conclusion

The main associated factors were shown to be time from purée preparation to use (> 24 hours), use of certain vegetables (borage and chard), and breastfeeding. Nitrate levels in both vegetables implicated as etiological factors in acquired MHb are very high.

INTRODUCTION

Clinically, methemoglobinemia (MHb) is characterized by occurrence in a previously healthy patient of a generalized violet blue, brownish color frequently in sick looking child, with little hemodynamic impact. MHb occurs when the ferrous component (Fe^{2+}) of hemoglobin is oxidized by oxidants and converted into Fe^{3+}; under these conditions, hemoglobin or, in other words, MHb is unable to transport oxygen. This effect almost only occurs in infants because of the lower acidity of their stomach (which promotes growth of microorganisms able to reduce nitrate to nitrite), presence of fetal hemoglobin (more easily oxidized by nitrite), and existence of a certain deficiency of the enzymatic system that reduces methemoglobin (NADH-methemoglobin reductase), which is however highly effective in adults (1).

We report the clinical, epidemiological, and dietary characteristics of 78 cases of MHb seen at our hospital from 1987 to 2010 and their relationship to intake of vegetables, some of which had not previously be considered to represent a risk.

PATIENTS Y METHODS

Participants

Participants were prospectively recruited at the pediatric emergency room of Hospital Público de Navarra (the reference tertiary maternity hospital in our region, covering a population of approximately 600,000 inhabitants) over the past 23 years (January 1987 to December 2010). A total of 156 patients were enrolled, all of them aged 4-18 months.

Study Design

A case-control study was conducted with children diagnosed with MHb induced by dietary intoxication secondary to vegetable intake at our hospital. A specific clinical investigation was made in all cases to rule out MHb of another origin (contact with oxidant agents, use of nitrate-rich water, or hypersensitivity to cow milk proteins).

We hypothesize that some epidemiological factors and others related to the preparation technique and composition of homemade purees could influence in MHb development.

Seventy eight patients attending the emergency room for reasons other than MHb caused by dietary intoxication were selected as controls. All patients with any chronic disease or underlying condition which could influence nutritional status or dietary habits in any way were excluded. Controls were selected at random after obtaining informed consent. Participants were enrolled at the pediatric emergency room on randomly selected days for a period of six months until the total 78 patients were recruited. The study was assessed and approved by the Navarre ethics and clinical trial committee.

Because of the importance of biological variables such as age, which plays a determinant role in occurrence of this condition, controls were selected based on a stratified distribution mimicking the distribution seen in descriptive analysis of the group of cases, so that there were no statistically significant age differences between cases and controls.

Data Sources

A system was designed to collect cases of MHb at the emergency room. This included a dietary survey and specific questions about the following variables: date of intoxication, type of feeding (breastfeeding only, bottle feeding, or both), ingredients of the last vegetable purée taken, time elapsed since preparation, and storage (refrigerator, freezer, or room temperature). Information was also collected about biological variables (sex, age, weight, and oxygen saturation), laboratory tests (percent methemoglobinemia, pH of venous blood), and treatments (administration and dose of methylene blue in mg).

A direct interview was performed to collect information about the following variables in controls: type of feeding (breastfeeding only, bottle feeding, or both), ingredients of the last vegetable purée taken, time elapsed since preparation, and storage (refrigerator, freezer, or room temperature). Information was also collected about biological variables, including sex, age, and weight.

Statistical Analyses

Nitrate tests in vegetables: These were performed at the food analysis laboratory of the Institute of Public Health of the Navarre Government using specific procedures recommended by the European Commission (2). Nitrate levels were measure in 135 samples of vegetables, obtained randomly from different distributors that supply food stores in our community. The previously homogenized sample was extracted in aqueous medium buffered with a saturated borax (boric acid) solution. Extracts were purified using a Sep-Pak C 18 cartridge. Nitrite and nitrate contents were tested by HPLC using an ion chromatography column and an ultraviolet detector at 214 nm. A calibration line was used for quantification. Automated calibration was performed between the limits of

30 mg/kg and 4000 mg/kg, while manual calibration was used for values higher than 4000 mg/kg.

Statistical analysis: SPSS for Windows v. 18 software was used for statistical analysis.

An alpha error of 0.05 and a beta error of 0.2 were used for sample size calculation.

A Student's t test for independent samples was used to compare means in quantitative variables, and a Chi-square test was used to compare proportions in dichotomous variables. A binary logistic regression test was used for multivariate analysis.

RESULTS

Descriptive study of cases.

The case-control study finally enrolled 156 subjects, 78 patients and 78 controls. Controls were randomly selected controlling distribution by age and sex. No statistically significant differences were found in the biological variables collected (Table 1), and both groups were therefore considered similar. Table 2 shows the characteristics of the 78 cases of MHb. Treatment used was methylene blue in 75/78 cases (96.2%), and no serious complications occurred.

Statistical comparison of differences between cases and controls in type of feeding (breastfeeding only or mixed feeding as compared to bottle feeding) provided the results shown in Table 3. Time elapsed from preparation to use was stratified into three groups: less than 24 hours (cases 9/78 [11.7%], controls 55/78 [70.5%]), 24 to 48 hours (cases 50/78 [64.9%], controls 12/78 [15.4%]), and more than 48 hours (cases 18/78 [23.4%], controls 11/78 [14.1%]). A statistical comparison was made of differences between cases and controls in food composition (types of vegetables used to prepare purée) (Table 3).

Multivariate study

A multivariate analysis was subsequently made to measure risk factors adjusted for development of diet-induced MHb using logistic regression. Table 4 summarizes the results, showing that breastfeeding, use of borage and chard, and a long time from preparation to use were risk factors, while a negative association was found for other vegetables (carrot, green bean, leek and zucchini).

Nitrate tests in vegetables

Nitrate levels were measure in 135 samples of vegetables. Results are given in Table 5. We compared our results to mean levels reported in Europe (Table 6)

DISCUSSION

MHb is not common in the pediatric population. Congenital MHb is very rare (3, 4), but MHb secondary to intake of or contact with potent oxidants may occur. Acquired MHb induced by drugs such as local anesthetics (5, 6), dapsone (7, 8), and anilines (9) has been reported. MHb has also been reported in cases of cow milk protein-sensitive enteropathy (10, 11). Intake of nitrate-rich water (12-15) or vegetables (16) could be a particular risk in infants under 12 months because of immaturity of their enzymatic systems and the low acid production capacity of their stomachs. Nitrates are converted into nitrites by the action of intestinal flora and are directly absorbed to blood flow (1, 15). It has important relevance in populations like ours, where prevalence of feeding with home made vegetables purees is high.

A series of 7 cases of MHb mainly related to beet intake was reported in a geographic area close to Navarre (16), and there are other reports related to intake of nitrate-rich vegetables in various geographic areas (17, 18). There have been in Spain isolated reports of MHb secondary to intake of chard purée (19, 20), but MHb has not been related to date with borage, a very characteristic vegetable in our environment. Cases of MHb related to nitrate-rich drinking water have also been reported (21), but this etiology was ruled out in this study.

We report the largest series of cases of MHb induced by vegetable consumption in infants, and think that there are several reasons for this. A clear factor associated to MHb was time from purée preparation to intake, in agreement with recent comments by Tamme y cols. (22) on the importance of time elapsed from preparation to use. Long time intervals between purée preparation and use and site of storage (room temperature) clearly promote conversion of nitrates to nitrites (15, 22, 23). Our series confirmed both factors to be associated to development of MHb. On the other hand, both chard and borage are leafy green vegetables with a greater tendency to accumulate nitrates (16). This study supported implication of chard and borage in MHb. Association of borage was particularly strong, with an OR = 5.293 (95% CI = 1.136-24.659), while chard was less strongly associated, with an OR = 2.004 (95% CI = 0.460-8.721). In our view, the most likely reason for loss of statistical significance for chard is that a lower amount of this as compared to borage is needed to prepare the same amount of purée, and borage also has higher nitrate levels (Table 5).

Testing of nitrate levels in vegetables from our region gives results consistent with those of epidemiological research on vegetables used to prepare purées, and highest levels are found in borage and chard (Table 5). High nitrate levels in chard had previously been reported (24). Nitrate accumulation in vegetables depends on temperature, sunlight, available nitrogen (groundwater), or mode of culture, among other factors (25). Navarre has been found to have nitrate-rich groundwater, and this may be a significant factor involved, indicating use of various nitrogen compounds in agriculture (26). In our region, it is customary to fertilize cereal fields with nitrate, which may possibly contaminate by filtration the areas where vegetables are grown. Another factor that may be related to the high incidence of MHb in our area is use of urea herbicides (27) in the setting of vegetable culture in small orchards, which is very common.

An additional reason for the high incidence of this condition is widespread consumption of homemade purées by the population. This habit, possibly related to the large production and wide availability of fresh vegetables, has been preserved in Navarre despite the fact that this is a region with a high economic development. In Spain, the upper valley of the Ebro river, including Navarre, La Rioja, and part of Aragon, is the area with the greatest borage consumption. The fact that less cases of MHb have been reported in the two last areas as compared to Navarre may in principle be related to climate (Navarre has less annual hours of sunlight and greater amounts of rainfall than La Rioja and Aragon) or other still unknown factors. In addition to the high nitrate levels in vegetables, preparation as a purée appears to increase nitrite levels (23).

The association of chard intake and MHb in Spain had already been reported by other authors (19, 20), and our group had suggested the potential implication of borage in MHb (28). However, this is the first study to show a clear relationship between borage intake and MHb.

The greater prevalence of breastfeeding in cases with MHb as compared to controls (Table 2) may be related to presence of nitrites in breast milk, and this mechanism may result in an increased intake of nitrates-nitrites by infants with MHb. In addition, conversion of nitrate to nitrite is enhanced by the lower stomach acidity in infants, which promotes growth of microorganisms capable of reducing nitrate to nitrite, a situation which may be influenced by breastfeeding (1,29). A relationship probably exists between the habit of preparing purées at home and breastfeeding in the sense that both variables may be associated to a more "natural" lifestyle. Other authors, however, have reported that breastfeeding protects against MHb in infants under 6 months of age (30). Mean age of our cases was 9.9 months, and neither data nor series are therefore comparable. It should also be noted that biological characteristics of our series greatly differ from those of cases reported in relation to drinking water in other reported series (14, 31).

Regulations for fresh vegetables and canned vegetables establish maximum tolerated contents in leafy green vegetables, particularly in lettuce and spinach (16, 32). However, these vegetables are seldom used to prepare purées for infants in our environment (Table 3). The Spanish Food Safety Agency recently recommended avoiding use of chard for preparing infant purées (33).

Despite all the data presented in this study the value of vegetable consumption and its proven benefits for health should be recognized (34). Some authors even suggest the potential benefits of a diet rich in nitrates and nitrites, mainly because of their influence on the cardiovascular system (35).

CONCLUSION

Our results indicate a very high incidence of MHb in our area due to intake of nitrate-rich vegetables, the significance of vegetables used for preparation of purées (chard and borage), the decisive role of time elapsed from purée preparation to use, and clear epidemiological evidence suggesting a higher frequency of breastfeeding in infants with MHb as compared to controls.

REFERENCES

1. Vittozzi L. Toxicology of nitrates and nitrites. Food Addit Contam 1992;9:579-85.
2. Opinion of the Scientific Panel on Contaminants in the Food chain on a request from theEuropean Commission to perform a scientific risk assessment on nitrate in vegetables,. The EFSA Journal (2008) Journal number, 689, 1-79. 2008.
3. Kedar PS, Warang P, Nadkarni AH, Colah RB, Ghosh K. A novel G143D mutation in the NADH-cytochrome b5 reductase gene in an Indian patient with type I recessive hereditary methemoglobinemia. Blood Cells Mol Dis 2008;40:323-7.
4. Arikoglu T, Yarali N, Kara A, et al. A novel L218P mutation in NADH-cytochrome b5 reductase associated with type I recessive congenital methemoglobinemia. Pediatr Hematol Oncol 2009;26:381-5.
5. Perez-Caballero Macarron C, Perez Palomino A, Moreno Fernandez L. [Probable methemoglobinemia following EMLA administration]. An Pediatr (Barc) 2005;63:179-80.
6. Shachor-Meyouhas Y, Galbraith R, Shavit I. Application of topical analgesia in triage: a potential for harm. J Emerg Med 2008;35:39-41.
7. Prasad R, Singh R, Mishra OP, Pandey M. Dapsone induced methemoglobinemia : Intermittent vs continuous intravenous methylene blue therapy. Indian J Pediatr 2008;75:245-7.
8. Williams S, MacDonald P, Hoyer JD, Barr RD, Athale UH. Methemoglobinemia in children with acute lymphoblastic leukemia (ALL) receiving dapsone for pneumocystis carinii pneumonia (PCP) prophylaxis: a correlation with cytochrome b5 reductase (Cb5R) enzyme levels. Pediatr Blood Cancer 2005;44:55-62.
9. Mauskar A, Karande S, Kulkarni M. Acquired methemoglobinemia due to contaminated colours: a preventable disaster. J Trop Pediatr 2009;55:139-40.
10. Hwang JB, Lee SH, Kang YN, Kim SP, Suh SI, Kam S. Indexes of suspicion of typical cow's milk protein-induced enterocolitis. J Korean Med Sci 2007;22:993-7.
11. Wirth S, Vogel K. Cow's milk protein intolerance in infants with methaemo globinaemia and diarrhoea. Eur J Pediatr 1988;148:172.
12. Ayebo A, Kross BC, Vlad M, Sinca A. Infant Methemoglobinemia in the Transylvania Region of Romania. Int J Occup Environ Health 1997;3:20-29.
13. Abu Naser AA, Ghbn N, Khoudary R. Relation of nitrate contamination of groundwater with methaemoglobin level among infants in Gaza. East Mediterr Health J 2007;13:994-1004.
14. Ward MH, deKok TM, Levallois P, et al. Workgroup report: Drinking-water nitrate and health--recent findings and research needs. Environ Health Perspect 2005;113:1607-14.
15. Fewtrell L. Drinking-water nitrate, methemoglobinemia, and global burden of disease: a discussion. Environ Health Perspect 2004;112:1371-4.
16. Sanchez-Echaniz J, Benito-Fernandez J, Mintegui-Raso S. Methemoglobinemia and consumption of vegetables in infants. Pediatrics 2001;107:1024-8.
17. Savino F, Maccario S, Guidi C, et al. Methemoglobinemia caused by the ingestion of courgette soup given in order to resolve constipation in two formula-fed infants. Ann Nutr Metab 2006;50:368-71.

18. Arranz L, Alustiza E, Ruiz Benito C, Angulo P, Perez-Yarza EG. [Acquired methemoglobinaemia (author's transl)]. An Esp Pediatr 1981;15:482-7.
19. Gomez Lumbreras A, Solaz Moreno L, del Villar Rubin S. Intoxicacion por pure de acelgas. An Pediatr (Barc) 2008;69:290-1.
20. Laporta Baez Y, Goni Zaballo M, Perez Ferrer A, Palomero Rodriguez MA, Suso B, Garcia Fernandez J. Metahemoglobinemia asociada a la ingestion de acelga. An Pediatr (Barc) 2008;69:191-2.
21. Sadeq M, Moe CL, Attarassi B, Cherkaoui I, Elaouad R, Idrissi L. Drinking water nitrate and prevalence of methemoglobinemia among infants and children aged 1-7 years in Moroccan areas. Int J Hyg Environ Health 2008;211:546-54.
22. Tamme T, Reinik M, Pussa T, Roasto M, Meremae K, Kiis A. Dynamics of nitrate and nitrite content during storage of home-made and small-scale industrially produced raw vegetable juices and their dietary intake. Food Addit Contam Part A Chem Anal Control Expo Risk Assess;27:487-95.
23. Chung JC, Chou SS, Hwang DF. Changes in nitrate and nitrite content of four vegetables during storage at refrigerated and ambient temperatures. Food Addit Contam 2004;21:317-22.
24. http://www.osakidetza.euskadi.net/r85-ckpubl02/es/contenidos/informacion/sanidad_alimentaria/es_1247/adjuntos/vigila9513.pdf.Visitado Julio 2011.
25. Bosch N. MJyPM. Influencia del tipo de abono sobre la acumulación de nitrato en vegetales. Anales de Bromatología 1993;XLIII-2/3:215-220.
26. http://www.crana.org/themed/crana/files/docs/197/082/situacion_navarra_nitratos_pesticidas.pdf. Visitado Julio 2011.
27. Watt BE, Proudfoot AT, Bradberry SM, Vale JA. Poisoning due to urea herbicides. Toxicol Rev 2005;24:161-6.
28. Herranz M, Clerigue N. Intoxicaciones en niños.. Metamoglobinemia. An Sist Sanit Navar 2003;26 Suppl 1:209-23.
29. Iizuka T, Sasaki M, Oishi K, Uemura S, Koike M, Shinozaki M. Non-enzymatic nitric oxide generation in the stomachs of breastfed neonates. Acta Paediatr 1999;88:1053-5.
30. Zeman CL, Kross B, Vlad M. A nested case-control study of methemoglobinemia risk factors in children of Transylvania, Romania. Environ Health Perspect 2002;110:817-22.
31. Avery AA. Infantile methemoglobinemia: reexamining the role of drinking water nitrates. Environ Health Perspect 1999;107:583-6.
32. EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on possible health risks for infants and young children from the presence of nitrates in leafy vegetables. EFSA Journal 2010;8(12):1935. [42 pp.] doi:10.2903/j.efsa.2010.1935.Available online: www.efsa.europa.eu/efsajournal.htm 2010.
33. http://www.aesan.msc.es/AESAN/web/rincon_consumidor/subseccion/nitratos_hortalizas.shtml. Visitado Julio 2011. 2011.
34. Katan MB. Nitrate in foods: harmful or healthy? Am J Clin Nutr 2009;90:11-2.
35. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 2009;90:1-10.

TABLE 1. BIOLOGICAL VARIABLES IN CASES AND CONTROLS. Table 1. Descriptive analysis of biological variables in cases and controls. [a]Results are given as males compared to total sample and proportion of males. [b]Result of probability in a Chi-square test for difference of proportions between cases and controls. [c]Results are given as mean and standard deviation. [d]Result of probability in a Student's t test for independent samples between cases and controls

TABLE 2. ANALYTICAL CHARACTERISTICS OF CASES. Table 2: Descriptive values of analytical variables

TABLE 3. NUTRICIONAL VARIABLES IN CASE AND CONTROLS (TYPE OF FEEDING, COMPOSITION, TIME AND TYPE OF STORAGE). Table 3. Descriptive analysis of purée composition in cases and controls. The values of simple binary logistic regression are expressed by coefficient B, p values, OR values and CI95%. Every variables is compared to the rest, except time elapsed from purée preparation to use, where less than 24 hours group is used as reference category. NC: In these variables the results are not avaible because in one group the number of patients is cero, so probability and OR values are not calculable

TABLE 4. MULTIVARIATE ANALYSIS OF VEGETABLES USED TO PREPARE PURÉE AT HOME AND BREASTFEEDING. Table 4. Multivariate analysis of risk factors for methemoglobinemia

TABLE 5. NITRATE LEVELS IN VEGETABLES. Table 5. Nitrate levels in vegetables (mg/kg)

TABLE 6. MEAN NITRATE LEVELS IN VEGETABLES COMPARED TO EFSA LEVELS. Table 6. [a]EFSA: European Food Safety Authority. [b]Figures are mean nitrate levels in mg/kg.

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Table 1

	Cases	**Controls**	**Sig.(p)**
Sex[a]	40/78 (51.3%)	45/78 (57.7%)	0.52[b]
Age[c] **(months)**	9.99 (3.94)	10.56 (3.02)	0.30[d]
Weight[c] **(Kg)**	9.02 (1.68)	8.94 (1.81)	0.79[d]

Table 2

	Mean	Minimum	Maximum	SD
% methemoglobin	31.7	8.7	61.3	11.9
% oxygen saturation	81.7	30	95	11.3
blood pH	7.37	6.99	7.45	0.07

Table 3

	B	P (sig.)	ExpB(OR)	95% CI
Breastfeeding	1,109	0,001	3,032	1,577 – 5,830
Carrot	-2,191	<0,001	0,112	0,049 – 0,255
Zucchini	-3,068	<0,001	0,047	0,019 – 0,116
Green bean	-1,665	<0,001	0,189	0,093 – 0,385
Borage	1,116	0,001	3,053	1,554 – 5,995
Chard	0,999	0,003	2,714	1,391 – 5,298
Spinach	0,000	1,000	1,000	0,196 – 5,114
Cabbage	-0,706	0,568	0,494	0,044 – 5,557
Cauliflower	n.c*	n.c*	n.c*	n.c*
Cardoon	n.c*	n.c*	n.c*	n.c*
Red cabbage	n.c*	n.c*	n.c*	n.c*
Leek	-2,071	<0,001	0,126	0,061 – 0,262
Time elapsed from purée preparation to use (%)				
24-48 hours	3,327	<0,001	25,463	9,894 – 65,532
> 48 hours	2,303	<0,001	10,000	3,573 – 27,989
Purée storage after preparation (%)				
Room temperature	1,297	0,012	3,659	1,338 – 10,010
Refrigerator	0.188	0,665	1,207	0,515 – 2,828
Freezer	-2,778	0,009	0,062	0,008 – 0,492

Table 4

	B	**P (sig.)**	**ExpB(OR)**	**95% CI**
Constant	1.909	0.100	6.744	
Breastfeeding	2.348	0.007	10.469	(1..916-57.205)
Carrot	-3.453	0.001	0.032	(0.004-0.228)
Zucchini	-3.076	< 0.001	0.046	(0.008-0.255)
Green bean	-1.908	0.018	0.148	(0.031-0.720)
Chard	0.695	0.354	2.004	(0.460-8.721)
Borage	1.666	0.034	5.293	(1.136-24.659)
Leek	-2.599	0.001	0.074	(0.017-0.330)
Time				
24-48 hours	2.861	< 0.001	17.477	(3..539-86..317)
> 48 hours	3.217	0.002	24.953	(3..318-187.663)

Table 5

Vegetable tested	Mean	N	Std. Deviation	Minimum	Maximum
Lettuce	1468..516	31	1544..936	45.00	6861.00
Spinach	915.857	7	369.573	259.00	1442.00
Chard	2811.647	17	1797..508	30.00	4974.00
Borage	3968.800	15	2630..340	394.00	9888.00
Leek	116.250	16	204.085	30.00	847.00
Carrot	56..333	18	45.099	30.00	207.00
Green bean	463.714	14	294.271	30.00	1226.00
Zucchini	591.375	8	248.507	143.00	1025.00
Frozen spinach	666.777	9	444.0618	270.00	1464.00
Total	1328.614	135	1811..946	30.00	9888.00

Table 6

Vegetable	EFSA (2008)(16)	Navarre
Chard	1690[b]	2811
Borage	Not available	3968
Lettuce	1324	1468
Spinach	1066	915
Leek	345	116
Carrot	295	56
Green bean	323	463
Zucchini	416	591

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