



Consumption of Cruciferous Foods, Ingestion of Glucosinolates and Goiter in a Region of Eastern Algeria

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Abstract

This study is about the estimation of the consumption of cruciferous vegetables, and the search for a possible relationship between the ingestion of glucosinolates provided by these foods and the emergence of endemic goiter. A prospective cohort survey was carried out on 1098 subjects residing in the district of EL-MILIA (Algeria) where endemic goiter is known to estimate the consumption of crucifers. Total glucosinolates levels in cruciferous vegetables, cabbage, cauliflower, fresh and cooked turnip and radish were determined by the glucose release method. The goiter was detected by clinical investigation. Both various fresh and cooked cruciferous vegetables have the same total glucosinolates levels as reported in other studies. The prevalence of endemic goiter was 17.5 %. The average consumption of crucifers was 380.30 g per person a week, i.e. 54.32 g per person a day, and the weekly ingestion of glucosinolates per person was 369.4 μ moles, or 52.7 μ moles per day. No dependence was observed between the consumption of glucosinolates and the disease in general. However, isolated stage 2 and 3 of goiter were dependent on the consumption of glucosinolates, stage 1 was not related to the disease. Despite the current consumption, at EL-MILIA, we estimate that glucosinolate's ingestion does not cause goiter, but may worsen the condition of subjects already affected.



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Introduction


Endemic goiter in Algeria is a significant health problem. However, iodine deficiency as the etiological factor is incompletely demonstrated

so far.¹ Many authors report that the variation in the prevalence of goiter in regions (Venezuela, Ecuador, New Guinea and Pisticulla) with a similar degree of iodine deficiency indicates that other causes

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are involved.^{2,3} These are natural goitrogens found in foods, geological sediments, synthetic products and water polluted by certain microorganisms.⁴

Natural goitrogens are probably the main cause in some regions where iodine intake is satisfactory. On the other hand, it appears that iodine deficiency in many regions is only a permissive factor, while natural goitrogens are the significant determinants in the prevalence of endemic goiter.⁵

Cruciferous vegetables are common components of the diet and have both beneficial and potentially harmful health effects. Following enzymatic degradation, some glucosinolates in these vegetables produce sulforaphane, phenethyl, and indolylic isothiocyanates that possess anticarcinogenic activity.⁶ In contrast, progoitrin and indolylic glucosinolates degrade to goitrin and thiocyanate, respectively, and may decrease thyroid hormone production.⁷

However, the existing results were obtained mainly from observations or experiments performed on animals.^{8,9} To date, there are insufficient data on the quantitative consumption of cruciferous vegetables required to cause thyroid dysfunction for the humans.¹⁰

The objective of this study is to investigate the consumption of foods from the cruciferous family, as well as the possible relationship between the ingestion of glucosinolates in these foods and endemic goiter.

Methods

Sample Collection

The vegetables studied are known to contain glucosinolates and belong to the cruciferous family.¹¹ We chose those of common consumption and found in abundance in the markets. Samples of cabbage, cauliflower, radish and turnip were obtained from the market of EL-MILIA which is supplied with goods from local and surrounding agricultural areas. We made five samples of 2 Kg each time, spaced of two days at the level of various points of the market. The different batches of the same variety are mixed, packed in cardboard, transported within 3 hours to the analysis laboratory (LNTA, UC1) and are kept in the refrigerator (4°C) before their analysis during the day.

Analysis of Total Glucosinolates

The determination of total glucosinolates is based on the assessment of glucose released after enzymatic hydrolysis (myrosinase 4UE/ml) of glucosinolates on mini-column (DEAE Sephadex A25 gel). Glucose is titrated after conversion to glucose-6-phosphate and subsequent reduction of Nicotinamide Adenine Dinucleotide Phosphate (NADP) to reduced NADP (NADPH). NADPH in turn reduces phenazine metho sulphate (PMS). The resulting reduced PMS will reduce idonitrotetrazolium chloride (INT) to reduced INT, which is measured colorimetrically at 520 nm.¹² The colorimetric response is proportional to the glucose concentration. Foods are analyzed in the raw state and after cooking in boiling water for 15-20 min, except the radish was consumed only in the fresh state.

Study Participants

The study was performed in the region of EL-MILIA where endemic goiter is recognized.¹³ This prospective cohort survey, took place at the level of the endocrinology department from November 2020 to July 2021, period when the concerned foods (crucifers) are available. The subjects included were 1098 persons including 491 males and 607 females aged from 22 to 45 years attending the specialist consultation for suspected thyroid disorders.

Clinical Screening for Goiter

The subjects included were examined for the presence of a goiter and are classified according to the standard guidelines of World Health Organization (WHO) (1979), i.e. stage 0: no goiter, stage I: palpable but not visible goiter with the head in hyper extension, stage II: visible goiter with the head in normal position, stage III: goiter visible at a distance.¹⁴

The research protocol was approved by the scientific council of the University Mentouri Constantine 1 Algeria (No: 03/2020 dated 26 January 2020), following the ethical principles of medical research in accordance with the Declaration of Helsinki (2013). Written informed consent was obtained from all subjects before their inclusion in the study.

Consumption of Cruciferous Foods

of the total population (1098 individuals) studied for goiter, 430 individuals agreed to complete the food

consumption data collection form. The subjects with goiter are all part of this sample. The person is asked about his or her food consumption in general and that of turnips, radishes, cabbage and cauliflower in particular. A food consumption data collection form is given to the consultants who are asked to fill it out daily and to come in every week for the follow-up survey to ensure the reliability of their answers and the motivation to follow study. A number of 668 subjects did abandon the current survey after goiter screen without giving reasons.

Table 1: Total glucosinolate content of fresh and cooked vegetables (μ moles / 100g)*

Vegetables	Fees Average \pm SD	Cooked Average \pm SD
Cabbage	113.70 \pm 4.60	42.00 \pm 1.60
Cauliflower	104.10 \pm 4.20	101.50 \pm 4.00
Turnip	188.10 \pm 7.50	115.80 \pm 5.10
Radish	256.00 \pm 10.20	/

*Glucosinolate content estimated in percent of fresh matter; SD: Standard deviations.

The glucosinolates ingestion extend of the subjects was divided into 13 classes of equal intervals to cover all the ingestion levels observed.

Statistical Analysis

Statistical analyses were done using XL STAT version 2009.1.01 (Add in soft 1995-2009, USA). The results of this study are presented using descriptive statistics such as arithmetic mean, standard deviation and frequency.

The dependence of the presence of goiter on glucosinolate intake is investigated by means of the χ^2 test at 12 d.d.l. for series < 30 . The reduced range is used instead of the χ^2 for $n > 30$. For the cases where there is dependence, we calculated the correlation coefficient (r). The significance level is less than 0.05.

Results

Total glucosinolates

The analysis of fresh and cooked vegetable cultivars for total glucosinolate content is reported in Table 1. The values related to the edible part of the plant are expressed in molar concentrations (micromoles glucosinolates / 100g fresh matter). Radish and turnip have the highest content of glucosinolates. Cooked vegetables, show a loss of about 61.6% in turnip and 63.0% in cabbage. Cauliflower is relatively stable in this treatment, it loses only 2.5%.

Table 2: Distribution of goiter according to size and appearance (n = 193)

Type of goiter	Number (n)	Percentage (%)	
According to the size	Stage 1	99	51.2
	Stage 2	70	36.2
	Stage 3	24	12.4
According to the aspect	Nodular	45	23.3
	Diffused	148	76.6

Table 3: Average consumption of crucifers per week and month

Vegetable	Quantity consumed in one week (g, %)		Quantity consumed in one month (g, %)	
	Average \pm SD(g)	Percentage (%)*	Average \pm SD(g)	Percentage (%)*
Cabbage	80 \pm 30	26.9	350 \pm 100	27.0
Cauliflower	130 \pm 20	44.4	570 \pm 100	44.3
Turnip	70 \pm 0.00	24.5	320 \pm 30	24.8
Radish	12 \pm 0.00	4.0	50 \pm 0.00	3.8
Total crucifers	297 \pm 20	100	1300 \pm 20	100

* Percentage of total consumption of all crucifers; SD: Standard deviations.

Table 4: Consumption class of vegetables (g) and glucosinolates (µmoles) in one week per person

Class1	Total population (n=430)										
	Num ber	Frequ-ency ²	Glucosinolate class average	Vegetable class average	Glucosinolate (µmoles;%)	Vegetable (g;%)	Cauliflower ³	Turnip ³	Radish ³	Glucosinolate (µmoles;%)	Vegetable (g;%)
50 - 100	68	15.8	60	67.9	9 (15.0)	21.4 (31.5)	27 (45.0)	25.9 (38.1)	24 (40.0)	20.6 (30.3)	0.0 (00.0)
100 - 150	66	15.3	121	140.6	20 (16.5)	47.6 (33.8)	61 (50.4)	58.6 (41.6)	40 (33.0)	34.4 (24.4)	0.0 (00.0)
150 - 200	71	16.5	180	186.8	21 (11.6)	49.9 (26.7)	92 (51.1)	48.4 (47.3)	47 (26.1)	40.5 (21.7)	20 (11.1)
200 - 250	50	11.6	220	226.1	25 (11.3)	59.5 (26.3)	110 (50.0)	105.7 (46.7)	59 (26.8)	50.8 (22.4)	26 (11.8)
250 - 300	37	8.6	272	279.7	32 (11.7)	76.1 (27.2)	130 (27.7)	124.9 (44.6)	76 (27.9)	64.5 (23.4)	34 (12.5)
300 - 350	20	4.6	315	321.3	35 (11.1)	83.3 (25.9)	157 (49.8)	150.9 (46.9)	83 (26.3)	71.5 (22.2)	40 (12.6)
350 - 400	19	4.4	368	372.0	42 (11.4)	100.0 (26.8)	171 (46.4)	164.4 (44.1)	100 (27.1)	86.2 (23.1)	55 (14.9)
400 - 450	11	2.5	420	438.8	54 (12.8)	128.5 (28.2)	186 (44.2)	178.8 (40.7)	130 (30.9)	112.0 (25.5)	50 (11.9)
450 - 500	17	3.9	465	484.1	59 (12.6)	140.4 (29.0)	200 (43.0)	192.3 (39.7)	150 (32.2)	129.3 (26.7)	56 (12.0)
500 - 550	22	5.1	529	535.3	63 (11.9)	149.9 (27.9)	217 (41.0)	208.6 (38.9)	170 (32.1)	146.5 (27.3)	79 (14.9)
550 - 600	18	4.1	571	582.8	65 (11.3)	154.7 (26.5)	260 (45.5)	250.0 (42.8)	174 (30.4)	150.0 (25.7)	72 (12.6)
600 - 650	15	3.4	617	627.5	67 (10.8)	159.5 (25.4)	305 (49.4)	293.2 (46.7)	168 (27.2)	144.0 (23.0)	77 (12.4)
650 - 700	16	3.7	665	681.0	75 (11.2)	178.5 (26.2)	312 (46.9)	300.0 (46.9)	200 (30.0)	172.4 (25.3)	78 (11.7)

1: classes correspond to vegetables (g) and glucosinolate (µmoles) ; 2 : percentages in relation to the total population ; 3 : For each vegetable, the percentages relative to the class average of glucosinolate and of this vegetable are mentioned, as well as the quantities in grams (vegetables) or in umoles (glucosinolate).

Table 5: Frequency of goiter by stage and glucosinolate consumption class (μ moles/person/week)

Total population (n=430)		Goitreous subjects (n=193)		Stage 1 (n=99)		Stage 2 (n=70)		Stage 3 (n=24)		
Class	Number	Frequency ¹	Number	Frequency ¹	Number	Frequency ²	Number	Frequency ²	Number	Frequency ²
50 - 100	68	15.8	24	35.2	19	79.1	5	20.8	0	0.0
100 - 150	66	15.3	25	37.8	18	72.0	7	28.0	0	0.0
150 - 200	71	16.5	27	38	19	70.3	6	22.2	2	7.4
200 - 250	50	11.6	20	40	13	65.0	5	25.0	2	10.0
250 - 300	37	8.6	16	43.2	8	50.0	6	37.5	2	12.5
300 - 350	20	4.6	11	55	6	54.5	4	36.3	1	9.0
350 - 400	19	4.4	10	52.6	5	50.0	4	40.0	1	10.0
400 - 450	11	2.5	6	54.5	2	33.3	3	50.0	1	16.6
450 - 500	17	3.9	10	58.8	3	30.0	5	50.0	2	20.0
500 - 550	22	5.1	14	63.6	2	14.2	8	57.1	4	28.5
550 - 600	18	4.1	11	61.1	2	18.1	6	54.5	3	27.2
600 - 650	15	3.4	9	60.0	1	11.1	5	55.5	3	33.5
650 - 700	16	3.7	10	62.5	1	10	6	60	3	30.0
Total	430	100	193	44.8	99	51.2	70	36.2	24	12.4

Frequency¹ (%): Frequency in relation to the corresponding total number of people ; Frequency² (%): Frequency in relation to the number of goiter patients in the corresponding class.

Prevalence of Goiter

The goiter screening included 1098 individuals, 491 of whom were males and 607 females. We recorded 193 cases of goiter in this study population, a frequency of 17.5%. The distribution of goiter according to size and appearance in goiter subjects is presented in Table 2.

Consumption of Crucifers and Ingestion of Glucosinolates

The average consumption of crucifers was 380.30 g per person in one week or 54.32 g per person in one day. In Table 3, the average intake of crucifers studied by all subjects in one week and one month are presented. In general, cauliflower

takes the first place followed by cabbage and turnip, then comes the radish.

The numbers for each class of vegetable(g) and glucosinolate (μ moles) intake per person in a week and the contribution of each vegetable to the overall cruciferous and glucosinolate intake are shown in Table 4. On average, the glucosinolate intake was 369.4 μ moles per person per week, or 52.7 μ moles per day with a range of 50 to 700 μ moles per week. At first sight, we observe that the participation rates of vegetables and glucosinolate contents correspond to each other in the same class. Except for cauliflower, where its minimum consumption is observed in the 50-100 class, while the corresponding glucosinolates are present in the lowest percentage in the 500-550 class. Furthermore, there is no relationship between the overall consumption at the class level and the participation rate of the vegetables in this same class. Generally, the highest or lowest contributions of each vegetable (or its glucosinolates) are not observed within the same class. However, radish and turnip participate with their minimum rate within the same class (150-200).

Glucosinolate Consumption and Different Stages of Goiter Evolution

We present in Table 5 the 13 classes of glucosinolate consumption (μ moles) and the different stages as well as the numbers of goitreous and non-goitreous. At first glance, we find that glucosinolate intake and disease frequency move in the same direction. An intake of 50 to 300 μ moles of glucosinolates per week corresponds to a goiter rate varying from 35.2 to 43.2%, beyond this consumption threshold and up to 700 μ moles, the frequency increases and can reach 63.6%.

The study of the hypothesis of dependence between the intake of glucosinolates and the disease in general without consideration of stage showed that these two variables were independent ($P > 0.05$). Concerning the influence of each stage in isolation, for stage 1 we did not see any dependence at the 5% probability level. On the other hand, stages 2 and 3 depend on the consumption of glucosinolates ($P = 0.01$; $P = 0.001$). Stage 3 was more strongly associated than stage 2. The cases of dependence obtained are confirmed by means of the linear correlation coefficient (r), so we found that stages 2 and 3 were positively correlated

with the consumption of glucosinolates ($r = 0.35$, $P < 0.05$; $r = 0.41$, $P < 0.05$).

Discussion

The average amount of total glucosinolates estimated in fresh cabbage is 113.7 μ moles /100g fresh matter. He *et al.*(2003) report levels of 421 and 429 μ moles glucosinolates /100g fresh matter. in Chinese cabbage and kale respectively.¹⁵ Our sample of cooked cabbage, contains an average of 42.0 μ moles glucosinolates /100g fresh matter. or about 37% of the total concentration of fresh. In cabbage and Brussels sprouts, losses due to cooking are 28.0 and 46.0%, respectively.¹⁶ For these different cultivars, authors have recorded losses of glucosinolates due to cooking of 40-80%.^{17,18}

For cauliflower in the study area, this vegetable contains an average of 104.1 μ moles glucosinolates /100g fresh matter. Mullin *et al.* (1977) reported an average of 105 μ moles glucosinolates /100g fresh matter for five varieties of cauliflower.¹⁹ Cooked cauliflower has a content of 101.5 μ moles glucosinolates /100g fresh matter, close to that of fresh. However, Sones *et al.*(1984) estimated this loss by cooking at 31%.¹⁶

The analysis of turnips in the present study, shows that the average amounts of total glucosinolates are respectively, for fresh and cooked vegetables, 188.1 and 115.8 μ moles glucosinolates /100g fresh matter. We note that cooking caused the loss of about 38.4% of the total glucosinolates of these turnips. Francisco *et al.*(2007) observed an average glucosinolate content for fresh turnips of 129.9 μ moles glucosinolates /100g fresh matter and a drop of about 64.0% due to this treatment.²⁰

An average total glucosinolate content of 256.0 μ moles/ 100 g fresh matter is measured in local radish. Hrnčirik and Velisek (1997) report averages of 92.81 μ moles glucosinolates /100g fresh matter in black radish varieties and 70.97 μ moles glucosinolates /100g fresh matter in white radish.²¹ We note that the variety we analyzed has the highest total glucosinolate content.

According to Dekker *et al.* (2000) during cooking of cruciferous vegetables, glucosinolate content is altered due to partial or complete inactivation

of myrosinase, thermal or plant myrosinase-mediated degradation of glucosinolates, loss of enzyme cofactors, leaching of glucosinolates and their metabolites into the cooking medium, or volatilization or thermal degradation of metabolites.²² These changes are primarily influenced by the initial peeling, cutting of the vegetable, duration and method of cooking, the type of plant matrix and the extent of its cellular disruption, and the chemical structure of the glucosinolate precursors.

The difference between our results and those of other authors is expected. Genetic, environmental, agronomic factors and the part of the plant examined are well known to affect the glucosinolate composition. In addition, the analytical methods used and the inherent experimental errors must be considered.

In our study we observed an average daily consumption of 54.32 g of cruciferous vegetables. Results have been reported in a study on cruciferous vegetables intake (IARC, 2004) showing a variable consumption in Europe ranging from 5 to 30g/day and the highest consumption was reported in China, above 100g/day, while relatively low daily intakes of 15g or less are reported for South Africa and some South American countries.²³ We note that the overall consumption of crucifers is relatively high in the EL-MILIA region.

The average glucosinolate intake per person was 369.4 μ moles per week, or 52.7 μ moles per day. Glucosinolate intake in Great Britain is estimated by Sones *et al.* (1984) to be 165.9 μ moles per person in one day.¹⁶ According to these authors, it is possible that 5% of the British population ingests more than 450 μ moles of glucosinolates daily during the winter. In Canada, Mullin and Sahasrabudhe (1978) reported an average daily intake of 17.5 μ moles of glucosinolates.²⁴ Fenwick and Heaney (1983), believe that this average is an underestimate because of the analytical methods used and because these authors omitted any contribution from mustard paste in common use.²⁵

It appears that the average consumption of glucosinolates in EL-MILIA is three times lower than in Great Britain, however it is three times higher than in Canada.

The contribution of individual vegetables to glucosinolate intake within each consumption class is not the same as the overall vegetable coverage within a given class. This is because the glucosinolate contents of the different vegetables are variable. We indicate that the consumption of cruciferous vegetables, mainly turnip, is part of the culinary preparations in the study region to a much greater extent than in other regions of Algeria.

Similar to the finding noticed by other authors,^{3,28} In our study, we found that the consumption of glucosinolates and the frequency of the disease evolve in the same direction and the stages 2 and 3 of goiter were positively correlated with the consumption of glucosinolates. These results indicate that the level of glucosinolate intake is not associated with the development of the disease in general, but tends to worsen the condition of subjects already suffering from goiter.

To date there is insufficient data available to define the amount of cruciferous vegetable consumption necessary to cause thyroid dys function. In humans, authors using radio iodine have reported that 194 μ moles was the minimum amount of goitrogen required to decrease radioiodine uptake, however, a smaller amount ingested, 70 μ moles, did not cause any inhibition of uptake.²⁶

According to Pearce and Braverman (2009), thiocyanates derived after ingestion of glucosinolates interfere with iodine metabolism in the thyroid gland by reducing iodide uptake by stimulating iodide efflux or by replacing iodide with thiocyanate, resulting in an increase in the level of iodine excretion.²⁷ In addition, thiocyanate also increases the formation of an essentially insoluble iodinated thyroglobulin in the thyroid in an iodine-depleted state.²⁸ In addition, glucosinolates produce isothiocyanate derivatives that spontaneously react with thiourea-forming amino groups and transform as potent anti-thyroid derivatives that interfere with iodine organification and thyroid hormone formation by inhibiting thyroid peroxidase activity and their action is not antagonized by iodide administration.²⁹ According to Leko *et al.*,(2021) low circulating T4 and T3 levels stimulate Thyroid Stimulation Hormone(TSH) secretion by the pituitary gland by

a feedback mechanism and if this condition continues for a longer period of time, thyroid gland hypertrophy occurs or a goiter develops.⁵

Particular attention should be paid to the fact that the policy of salt iodization to combat iodine deficiency remains ineffective. Statistics are scarce and not very accurate. In 1992, nearly 90% of households used iodized salt compared to 50% in 2008.³⁰ We note the non-compliance of certain manufacturers with the iodization of food salt. On eight brands of salt analyzed in 2020 in our laboratory (LNTA) 25% contain a content <7 mg/Kg, 50% contain <50 mg/Kg and only 25% are in compliance with the regulation ≥ 50 mg/Kg.

Conclusion

In spite of the current consumption, in EL-MILLIA, we believe that the ingestion of glucosinolates does not cause goiter, but is likely to worsen the condition of subjects already affected.

It should also be remembered that the determination of glucosinolate intake is still not very accurate. This is due to the difference in content between plant species and within the cultivar itself, as well as the variability of intake between different ethnic

groups and different socio-economic and cultural levels. Also, other sources of glucosinolates are not taken into account, such as milk, poultry products, and vegetable oils.

This study should allow further research on natural goitrogens and their relationship with the goiter endemic in Algeria. The level of glucosinolate intake in risk groups and in various goiter-affected regions is to be determined. The fate and physiological effects of these compounds in the body are to be further investigated.

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Conflict of Interests

The authors declared that there is no conflict of interest regarding the publication of this paper.

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