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THE IMPACT OF DIETARY NITRATES AND ACRYLAMIDE INTAKE ON SYSTEMIC REDOX STATUS IN HEALTHY YOUNG ADULTS

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Abstract

Objectives: The nitrogen-containing xenobiotics, such as nitrates and acrylamide may potentially influence systemic redox status and contribute to the generation of oxidative stress (OS) in the human body, but there is still a lack of studies that would evaluate the various parameters assessing the oxidative-antioxidant balance. The aim of this study was to evaluate the exposure to nitrates and acrylamide derived from daily diet and to analyze the impact of these nitrate-containing xenobiotics on the parameters of systemic redox status in healthy young adults. **Material and Methods:** To assess nitrate and acrylamide intake in the study population, a semi-quantitative food frequency questionnaire was used. Systemic redox status was evaluated by measurement of a panel of biochemical parameters: enzymatic (glutathione S-transferase, glutathione reductase, glutathione peroxidase [GPx]) and non-enzymatic (uric acid, bilirubin and albumin), thiol/disulphide homeostasis parameters (total thiol, native thiol, and disulfide) and oxidative/antioxidant balance indicators (total antioxidant status, total oxidant status, OS index). **Results:** The average consumption of nitrates and acrylamide in the study population was 1.24 mg/kg b.w./day and 0.23 μg/kg b.w./day, respectively, which is within the normal value range. Of 12 measured parameters, significant differences were revealed for disulfide and total thiol levels, which were increased in the subgroup with the highest daily intake of nitrates compared to the subgroup with the lowest intake; for GPx, which was highest in the subgroup of the lowest daily intake of acrylamide; and for native thiols in the subgroup with the highest daily intake. **Conclusions:** The intake of nitrogen-containing xenobiotics within the range considered as normal does not markedly influence redox state parameters in healthy young adults. Some significant changes were revealed only for thiol/disulphide homeostasis parameters, which may be the first line of antioxidant defense, as well as for GPx activity. Compensative mechanisms in healthy young people are efficient enough to neutralize OS induced by slightly increased exposure to nitrogen-containing xenobiotics delivered with food. Int J Occup Med Environ Health. 2023;36(6):773–87

Key words:

acrylamide, nitrates, oxidative stress, intake with food, nutritional survey, oxidative/antioxidative balance

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INTRODUCTION

Maintaining redox balance, which is defined as the balance between oxidants (such as reactive oxygen, nitrogen and chlorine species) and counteracting defense systems (enzymatic and nonenzymatic), is important for overall human health [1]. When the antioxidant system is impaired or overwhelmed, the phenomenon of oxidative stress (OS) may occur [2]. Much of the literature data evidences that chronic OS has been linked to a number of diseases, including diabetes, cardiovascular disease, neurodegenerative disorders, autoimmune diseases or cancer [3,4]. Therefore, the assessment of systemic redox status can provide information on the biochemical condition of the human body in terms of harmful oxidative processes [2,5].

A healthy lifestyle, including a balanced diet, regular exercise, and avoidance of environmental toxins, can help support the body's natural detoxification and antioxidant systems and reduce the risk of OS-related diseases. On the other hand, diet is an important source of xenobiotics, which can generate oxidants and lead to OS if not properly metabolized and eliminated from the organism [4]. Although food-derived xenobiotics have received increasing attention in recent years, their role in oxidative/antioxidant homeostasis is underestimated and insufficiently investigated as they are a source of everyday exposure [6]. In particular, little is known about the disturbance of redox balance by food-derived xenobiotics containing nitrogen, e.g., nitrates and acrylamide [7,8].

Nitrates are naturally occurring compounds found in food, water and soil. Approximately 80% of dietary nitrates are derived from vegetable consumption; another source are processed meats, in which they function as preservatives [9,10]. Nitrates are converted to nitrites in the body, and then to nitric oxide (NO), which plays an important role in regulating blood flow, blood pressure, and immune function [11]. Nitrates are considered beneficial and safe for human health. However, their excessive consumption may lead to negative effects, such as formation of reactive nitrogen species (RNS), especially peroxynitrite, which can contribute to the development of OS; the formation of carcinogenic nitrosamines when nitrites react with amino acids in the stomach; or the induction of methemoglobinemia, which results in impaired oxygen transport to the body's cells and their hypoxia [12–14].

In turn, acrylamide is one of the most recognized harmful substances found in food. It is a chemical compound that is formed naturally in certain dietary products (rich in carbohydrates and low in proteins) during high-temperature cooking processes such as frying, baking, and roasting. It is found in high levels in French fries, potato chips, bread, and coffee. Tobacco smoke is another important source of acrylamide [15,16]. Acrylamide is metabolized in the organism to form glycidamide, which is a toxicological hazard. Acrylamide exposure has been linked to OS and associated with neurotoxic, genotoxic, carcinogenic effects and fertility disorders [17,18]. This xenobiotic has been classified by the International Agency for Research on Cancer (IARC) as group A2, i.e., as a compound probably carcinogenic for humans [19].

In the above aspects, a reliable evaluation of the sources of these compounds in dietas possible players in redox imbalance and OS generation is very important. The aim of this study was to assess the exposure to nitrates and acrylamide derived from daily diet among healthy young adults and to analyze the impact of their intake on the parameters of the systemic redox status. The exposure to nitrates and acrylamide was assessed by nutritional survey, and redox status was evaluated by measuring of the several parameters linked to oxidative/antioxidant balance such as: glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), total antioxidant status (TAS), total oxidant status (TOS), OS index (OSI), total thiol, native thiol, and disulfide

levels, uric acid, bilirubin and albumin concentrations. To the authors' knowledge, such research is still lacking in the scientific literature.

MATERIAL AND METHODS

Participants

The study group included 151 participants (89 women and 62 men) aged 19–26 years (M±SD 23.28±1.67), who were enrolled as students at Wroclaw Medical University, Wrocław, Poland. The inclusion criteria for the study group were: residency in Wrocław or its suburbs for min. 3 years, student status, general good health, absence of chronic diseases and no declared intake of medications. The exclusion criteria for the study group were: acute and chronic inflammatory conditions; chronic use of drugs, dietary supplements or addictive substances. The study protocol was approved by the Wroclaw Medical University Ethics Board, Poland (consent No. KB-808/2020 and KB-147/2022) and all subjects gave written informed consent.

Methods

Blood collection

Fasting blood samples were drawn into the tubes containing or not anticoagulant after the day of filled a questionnaire of nitrogen-containing compounds intake. Plasma and serum samples, respectively, were prepared by standard centrifugation of blood and stored in –80°C until measurements of examined parameters were performed.

Dietary intake assessment

All participants filled a semi-quantitative food frequency questionnaire (sFFQ) the day before blood was drown, to subsequently assess nitrate and acrylamide intake. The sFFQ consisted of 47 questions. Of them, 5 referred to gender, age, weight, height and smoking habits of participants. Other 42 questions were related to habitual consumption (in portions per day, week or month) of foods that are relevant sources of acrylamide (22 products) and nitrate (20 products) in diet. The portion size of the products was assessed using the *Album of photographs and dishes*, which was published by the Food and Nutrition Institute in Warsaw, Poland [20]. The amounts of dietary nitrates and acrylamide were calculated using published data on their content in foods originating in Poland, members of the European Union (EU), the UK and USA [9,16,17, 21–25]. For multiple data reported on the same foods, the following order of priority was applied:

- the reliability of the method used for measurement of nitrogen-containing compounds,
- the degree of representativeness of the data,
- the origin of the products analyzed.

Intake from individual foods was classified into respective food groups relevant in nitrate and acrylamide supply. To confirm the adequacy of sFFQ for assessing the intake acrylamide and nitrate, in the group of 24 participants (12 females and 12 males) the 7-day dietary recall was also performed and data calculated to cross-check the results gathered from sFFQ. Acrylamide median intake calculated using the 7-day food recall amounted to 97% (Q1–Q3: 71–207%); while nitrate median intake amounted to 102% (Q1–Q3: 80–132%). The correlation between results obtained in sFFQ and 7-day dietary recall were $r = 0.79$ ($p = 0.000$) and $r = 0.88$ ($p = 0.000$) for acrylamide and nitrate intakes, respectively. The data collected using sFFQ allowed for assessment of current dietary exposure to nitrogen-containing food in all 151 participants of the study. For the assessment of value of daily intake of nitrates (DIN) and daily intake of acrylamide (DIA), the data derived from sFFQ were analyzed and appropriate formulas were applied to calculate DIN and DIA, as described earlier [26].

Biochemical analyses

The systemic redox status was evaluated by measurement of a panel of biochemical parameters: enzymatic (GST,

GR, GPx) and non-enzymatic biomarkers (uric acid, bilirubin and albumin), thiol/disulphide homeostasis parameters (total thiol, native thiol, and disulfide) as well as oxidative/antioxidant balance indicators (TAS, TOS, OSI).

The activity of GST and concentration of GR and GPx were measured with appropriate commercial assay kits obtained from Sigma-Aldrich (Saint Louis, MO, USA), cat. No CS0410, or Cusabio (Houston, TX, USA), cat. No. CBS-E14286h and cat. No. CSB-EL009866HU, respectively.

The TAS value was measured by the spectrophotometric method with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), using a Randox TAS kit (Randox Laboratories, Crumlin, UK) and a Konelab 20i analyzer (ThermoSientific, Waltham, MA, USA), and results are expressed in mmol/l of Trolox equivalent [27]. The TOS was measured by the spectrophotometric determination of colored oxidized product using xylenol orange described [28]. In this method, the ability of serum oxidants to oxidize ferrous-ion-o-dianisidine complex is used. The results are expressed in μmol/l of H_2O_2 equivalent. Oxidative stress index was calculated as a TOS:TAS ratio, using the values of both parameters expressed in the given units [29].

Thiol/disulphide homeostasis parameters were measured according to Erel et al. [30]. Briefly, total thiol content was measured by reducing disulfide bonds by sodium borohydride, and native thiols were measured using Ellman method (without reduction). Then formaldehyde was added to remove unused sodium borohydride, and a reaction with DTNB (5,50-dithiobis-(2-nitrobenzoic acid) was performed. The disulphide level was calculated as half of the difference between total thiol and native thiol content.

Albumin concentration was measured by colorimetric method with bromocresol green; for uric acid, an enzymatic method with the specific oxidation of uric acid by uricase was used, and total bilirubin was examined by colorimetric method with diazotized sulfanilic acid. All commercial reagent kits were supplied by Thermo Electron Corporation, Vantaa, Finland, and applied on the Konelab 20i (Thermo Electron Corporation, Vantaa, Finland) analyzer in the Diagnostics Laboratory for Teaching and Research at Wroclaw Medical University, Wrocław, Poland.

Statistical analyses

Statistical analyses were performed using Statistica v. 13.0 PL (StatSoft Inc., Tulsa, USA). The normality of distribution of the variables was examined by the Shapiro-Wilk test. Descriptive statistics in subgroups based on sex, body mass index (BMI) value and smoking status as well as quartiles of DIN and DIA were made. The Mann-Whitney U test, ANOVA with Tukey *post hoc* test or Pearson's χ^2 test were used for subgroups comparisons, depending on the variable's character. The results were expressed as median and interquartile limits (Q1–Q3). A p-value <0.05 was considered as statistically significant.

RESULTS

Based on anthropometric measurements, BMI was calculated (M±SD 22.40±3.18). The majority of participants (76.2%) had a normal body weight, 22.6% were overweight and 8.7% underweight according to WHO classification [31]. Moreover, declared smoking status was recorded. Based on the collected data, it was found that 20.5% of volunteers declared a smoking habit.

The data of the DIN as well as DIA calculated on the basis of a completed sFFQ are presented in Table 1. Additionally, values of these parameters are presented in subgroups of study participants divided according to sex, BMI and smoking status.

According to obtained data, a significantly higher intake of acrylamide was observed in men (about 1.5 times) compared to women, but there were no significant differences in intake of nitrates between women and men. There were

Table 1. Daily intake of nitrates (DIN) and acrylamide (DIA) in study of 151 students at Wroclaw Medical University, Wrocław, Poland, examined in 2020–2022

Variable	DIN [µg/kg b.w./day] (Me (Q1–Q3))	p	DIA [µg/kg b.w./day] (Me (Q1–Q3))	p
Participants total (N = 151)	1238.78 (599.59–2505.23)	–	0.230 (0.133–0.404)	–
Sex		0.38		**0.019**
women (N = 89)	1299.03 (606.14–2679.70)		0.187 (0.127–0.372)	
men (N = 62)	1104.50 (558.18–2173.23)		0.288 (0.140–0.543)	
BMI		0.15		0.340
underweight (N = 10)	2739.38 (751.23–3680.52)		0.237 (0.155–0.406)	
normal weight (N = 115)	972.59 (523.99–2286.62)		0.230 (0.136–0.401)	
obesity (N = 26)	1503.70 (828.40–2619.77)		0.194 (0.090–0.404)	
Smoking status (no/yes)		0.96		**<0.001**
non-smoking (N = 120)	1240.49 (618.00–2505.23)		0.213 (0.133–0.399)	
smoking (N = 31)	1003.35 (457.40–2517.12)		0.400 (0.257–0.638)	

The Mann-Whitney U test was applied.
Bolded are p-values <0.05 (significant).

no significant differences in subgroups divided according to BMI value. The DIA values were almost twice as high in smokers than in non-smoking participants.

The main sources of daily food-derived nitrates and acrylamide, indicated by respondents in the completed nutritional survey, are presented in Figure 1 (1a – nitrates, 1b – acrylamide).

The main sources of nitrates indicated by respondents were: lettuce (37.3%) and beetroot (29.1%), followed by carrots (6.6%), cucumbers (6.2%) and arugula (6.0%). French fries (32.3%), crackers (16.3%), chips (12.0%) and gingerbread cookies (10.0%) were indicated as the main source of acrylamide uptake.

Table 2 presents the results of measurements of various parameters reflecting systemic redox status in the whole study group as well as in subgroups divided according to sex.

Statistically significant differences between sex-based subgroups were revealed only for the activity of GR, TOS value, uric acid and albumin concentration. The most significant difference ($p < 0.001$) was observed for uric acid (1.4 times higher in men compared to women).

The authors also analyzed the potential impact of the dietary intake of nitrates and acrylamide on the level of systemic redox status. Additionally, statistical analysis was performed in subgroups distinguished on the basis of increasing values (quartiles) of DIN and DIA and is shown in Table 3.

As shown in Table 3, total thiol and disulfide concentration showed a gradual increase in quartiles of nitrate intake, resulting in a statistically significant difference between Q1 and Q4 ($p < 0.022$ and $p < 0.016$, respectively). Also, significant differences were observed in GPx activity and native thiol values in relation to acrylamide intake. Higher intake of acrylamide resulted in a decrease of GPx concentration (Q3 and Q4 in comparison to Q1 and Q2) and an increase in the value of native thiol (the highest in Q3). This observation was confirmed by correlation analysis (DIA vs. GPx $r = –0.32$, $p < 0.001$ and DIA vs. native thiol value $r = 0.29$, $p < 0.001$). This

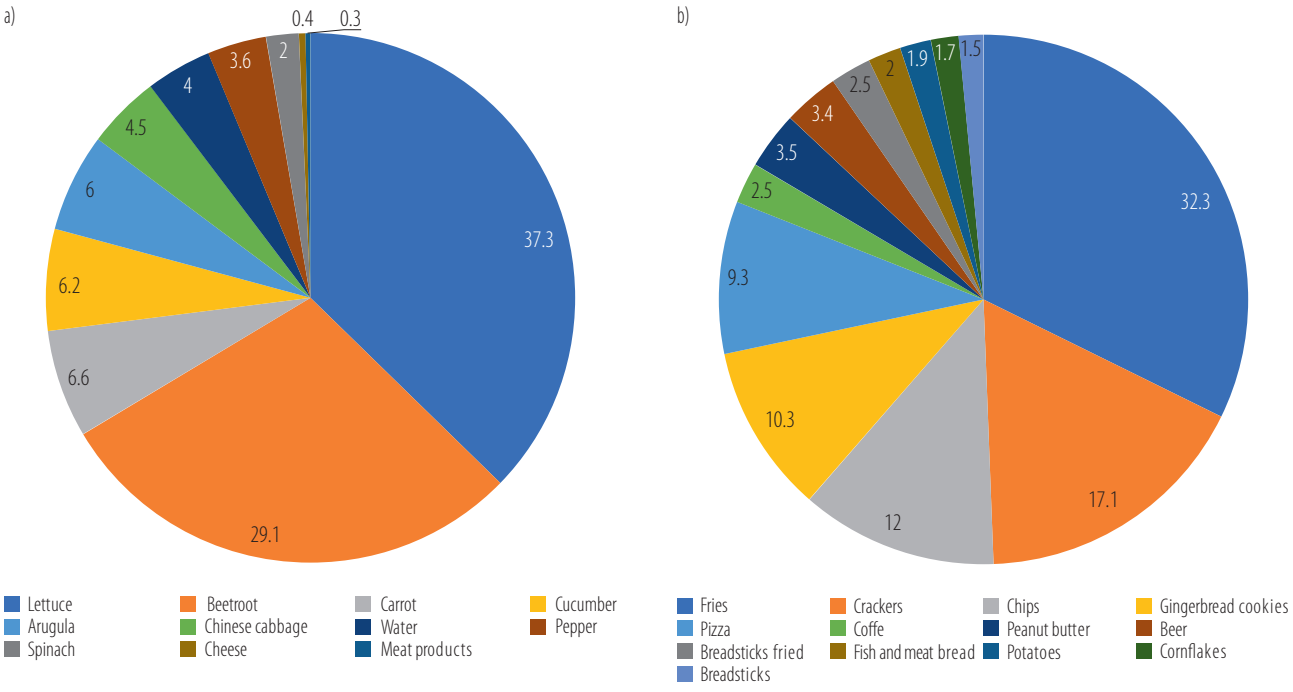

Figure 1. The contribution of foods to a) nitrate and b) acrylamide intake in the studied group of students at Wroclaw Medical University, Wrocław, Poland, examined in 2020–2022

relationship was more strongly expressed in the sex-based subgroups (DIA vs. GPx in men $r = -0.52$, $p < 0.001$ and DIA vs. native thiols in women $r = 0.28$, $p = 0.011$).

DISCUSSION

Due to the common occurrence of nitrates and acrylamide in the environment, people are extensively exposed to these xenobiotics. Literature data indicate that nitrates and acrylamide are mostly supplied to the organism with food, and their impact on the oxidative-antioxidant balance has been indicated [15,32]. To the authors' knowledge, there is no available database on dietary nitrates and acrylamide intake in Polish young adults in relation to their influence on systemic redox status, so it seemed interesting to investigate this issue deeper. Therefore, the authors assessed the amount of diet-derived nitrates and acrylamide in the population of students living in Wrocław, Poland. So far, average daily intake of nitrogen-containing xenobiotics have been examined in different age groups and nationalities [33,34]. In the authors' study, the daily consumption of nitrates and acrylamide was assessed based on the respondents' answers regarding the type, frequency and portion size of consumed products in the nutritional survey. According to the European Commission's and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the current acceptable daily intake (ADI) for nitrites and nitrates are 0.07 mg/kg b.w./day and 3.7 mg/kg b.w./day, respectively [35]. To date, there is still no acceptable DIA, however the European Food Safety Authority (EFSA) has determined a benchmark dose lower confidence limit (BMDL10) of 0.17 mg/kg b.w./day for carcinogenesis and 0.43 mg/kg b.w./day for neurotoxicity [36]. In a 2015 report, based on European data, EFSA estimated average acrylamide intake as ranging 0.4–1.9 μg/kg b.w./day [37]. It was showed that the main sources of nitrates were lettuce and beetroot (Figure 1a). These products are reported as some of the richest vegetable sources of nitrates, containing 1000–3000 mg/kg fresh

Table 2. The values of parameters of systemic redox status estimated in the study group of students, Wroclaw Medical University, Wrocław, Poland, examined in 2020–2022

Parameter	Participants (N = 151)			p
	total	women (N = 89)	men (N = 62)	
Glutathione S-transferase [U/ml] (Me (Q1–Q3))	61.99 (48.52–86.25)	67.38 (48.52–88.95)	59.30 (47.17–75.42)	0.196
Glutathione reductase [mU/ml] (Me (Q1–Q3))	16.81 (14.26–21.90)	16.81 (11.72–20.63)	19.36 (14.26–24.45)	**0.009**
Glutathione peroxidase [µU/ml] (Me (Q1–Q3))	114.03 (88.91–141.09)	112.10 (88.91–129.49)	116.93 (90.84–143.02)	0.581
Total antioxidant status [mmol/l Trolox] (Me (Q1–Q3))	1.85 (1.70–1.98)	1.84 (1.72–1.94)	1.88 (1.65–2.10)	0.234
Total oxidant status [µmol/l H_2O_2] (Me (Q1–Q3))	10.13 (6.54–13.25)	9.50 (6.02–12.66)	11.12 (8.23–13.29)	**0.034**
Oxidative stress index (Me (Q1–Q3))	5.58 (3.45–7.51)	5.34 (3.24–7.17)	6.10 (3.88–8.30)	0.056
Thiol [µmol/l] (Me (Q1–Q3))				
total	981.79 (761.15–1143.17)	1002.80 (741.30–1162.07)	930.54 (791.50–1110.66)	0.772
native	394.58 (306.787–454.12)	365.40 (306.09–437.78)	412.09 (314.10–465.79)	0.104
Disulphide [µmol/l] (Me (Q1–Q3))	567.36 (310.53–875.72)	5821.72 (331.54–876.40)	504.32 (310.53–809.16)	0.514
Uric acid [mg/dl] (Me (Q1–Q3))	4.69 (3.84–5.63)	4.06 (3.61–4.71)	5.70 (4.94–6.44)	**<0.001**
Bilirubin total [mg/dl] (Me (Q1–Q3))	1.20 (0.14–0.30)	0.19 (0.14–0.27)	0.21 (0.13–0.41)	0.249
Albumin [g/dl] (Me (Q1–Q3))	4.59 (4.45–4.70)	4.55 (4.42–4.69)	4.63 (4.50–4.73)	**0.029**

Mann-Whitney U test was applied.
Bolded are p-values <0.05 (significant).

Table 3. The values of parameters of systemic redox status estimated in subsequent quartiles of nitrates and acrylamide intake in 151 students at Wroclaw Medical University, Wrocław, Poland, examined in 2020–2022

Parameter	Daily intake of nitrates (Me (Q1–Q2))				p	Daily intake of acrylamide (Me (Q1–Q2))				p
	Q1 ≤164.66 (N = 38)	Q2 164.67–334.32 (N = 37)	Q3 334.33–675.37 (N = 38)	Q4 ≥675.38 (N = 38)		Q1 ≤134.29 (N = 37)	Q2 134.30–229.99 (N = 38)	Q3 230.00–403.99 (N = 38)	Q4 ≥404.00 (N = 38)	
GST [U/ml]	59.30 (51.21–86.25)	63.34 (40.43–80.86)	67.38 (51.21–86.25)	64.69 (45.82–88.95)	0.900	53.91 (43.13–70.08)	63.34 (48.52–99.73)	64.69 (51.21–94.34)	70.08 (51.21–91.64)	0.201
GR [mU/ml]	16.81 (14.26–19.36)	16.81 (11.72–21.90)	18.08 (11.72–25.72)	19.36 (14.26–24.45)	0.108	19.36 (14.26–29.54)	16.48 (11.72–19.36)	19.36 (14.26–21.90)	19.36 (11.72–21.90)	0.573
GPx [µU/ml]	117.90 (92.77–143.02)	111.13 (83.11–146.89)	119.83 (90.89–139.16)	108.23 (94.70–127.56)	0.930	123.69 (104.37–152.69)	119.83 (96.64–139.16)	109.20 (85.04–127.56)	101.47 (67.65–129.49)	**0.003** Q1 vs. Q4: **0.037**
TAS [mmol/l Trolox]	1.83 (1.69–2.00)	1.85 (1.70–1.99)	1.86 (1.69–1.99)	1.83 (1.74–2.00)	0.956	1.85 (1.74–1.94)	1.83 (1.71–1.92)	1.94 (1.69–2.04)	1.86 (1.70–2.02)	0.914
TOS [µmol/l H_2O_2]	10.13 (7.59–12.05)	10.33 (6.54–15.82)	10.84 (6.58–13.25)	8.86 (5.93–12.05)	0.646	8.86 (6.05–11.4)	10.3 (6.17–13.29)	10.48 (6.67–13.25)	10.69 (8.43–15.19)	0.339
OSI	5.64 (3.96–7.17)	5.29 (3.37–8.53)	6.22 (3.68–7.42)	5.15 (3.24–6.61)	0.380	4.94 (3.13–6.61)	5.60 (3.28–7.78)	5.84 (3.68–6.76)	5.66 (3.91–8.30)	0.410
Thiol [µmol/l]										
total	901.24 (670.09–1095.03)	967.91 (809.01–1162.07)	1001.63 (842.87–1140.11)	1043.66 (830.03–1262.34)	**0.048** Q1 vs. Q4: **0.023**	1046.99 (846.44–1204.97)	1078.74 (762.32–1190.00)	928.41 (791.50–1104.19)	856.88 (740.13–1110.66)	0.423
native	414.43 (330.95–454.12)	371.82 (299.80–454.12)	394.58 (347.80–465.78)	347.29 (301.84–442.45)	0.149	329.93 (253.32–414.43)	395.17 (313.58–436.61)	416.18 (301.84–459.96)	429.02 (353.42–473.97)	**0.005** Q1 vs. Q2: **0.025** Q1 vs. Q3: **0.0001** Q1 vs. Q4: **0.015**
Disulphide [µmol/l]	412.09 (246.32–738.17)	598.69 (349.05–876.40)	535.84 (326.87–825.84)	671.77 (384.08–1030.98)	**0.032** Q1 vs. Q4: **0.016**	708.61 (412.09–916.92)	625.15 (331.54–876.40)	505.49 (366.57–792.47)	430.77 (253.33–825.84)	0.214
Uric acid [mg/dl]	4.83 (4.08–5.50)	4.78 (3.71–5.91)	4.42 (3.67–5.55)	4.59 (3.70–5.53)	0.354	4.76 (4.14–5.38)	4.13 (3.56–4.83)	5.05 (3.68–5.91)	4.92 (3.84–5.63)	0.383
Bilirubin total [mg/dl]	0.18 (0.12–026)	0.22 (0.15–0.35)	0.22 (0.16–0.32)	0.19 (0.14–0.27)	0.188	0.22 (0.15–0.32)	0.19 (0.12–0.28)	0.22 (0.13–0.42)	0.19 (0.14–0.27)	0.870
Albumin [g/dl]	4.60 (4.43–4.70)	4.63 (4.43–4.85)	4.57 (4.47–4.65)	4.56 (4.49–4.70)	0.686	4.62 (4.51–4.73)	4.55 (4.41–4.67)	4.56 (4.45–4.67)	4.57 (4.36–4.75)	0.242

GPx – glutathione peroxidase; GR – glutathione reductase; GST – glutathione S-transferase; OSI – oxidative stress index; TAS – total antioxidant status; TOS – total oxidant status.
ANOVA with Tukey *post-hoc* test were applied.
Bolded are p-values <0.05 (significant).

weight [38]. The main source of acrylamide in the examined study group were French fries, crackers and chips. According to Mojska et al. [23], these are compounds with particularly high acrylamide content, 0.3–0.7 mg/kg of product. According to the obtained results, average consumption of nitrates and acrylamide in the study population (1.24 mg/kg b.w./day and 0.23 μg/kg b.w./day, respectively) is within the range of values considered as normal. The contribution of different food groups to nitrates and acrylamide intake in the examined student group stays in alignment with other studies and the authors' earlier examination [26]. Jackson et al. [36] indicated vegetables (mainly lettuce and spinach) as the main source of nitrates in the group of Australian women, and the average intake of nitrates in the diet was estimated as 65–70 mg/day [39]. On the basis of a 4-day nutritional survey conducted in Polish women (aged 19–26 years old), Wawrzyniak et al. [40] showed the average intake of $NaNO_2$/person/day as 1.4 mg and $NaNO_3$/person/day as 119.6 mg. The main sources of sodium nitrate III were meat products (52%) and cereal products (21.%), while sodium nitrate V was provided by vegetables and processed foods (82%), mainly cabbage (15%), potatoes (12%), lettuces (10%) and beets (9%). Other studies by Wawrzyniak et al. [41] assessed the dietary intake of sodium nitrates III and V in a group of Polish students aged 21–24 years, similar to the authors' study group, through a 3-day nutritional survey. The authors reported the average daily intake of sodium nitrates V as 77.3 mg $NaNO_3$/person/day, with no statistically significant differences depending on sex, which is consistent with the authors' study. The authors indicated the main sources of sodium nitrate III to be meat products (56.5%) and cereal products (20%), and sodium nitrate V was derived primarily from vegetables and vegetable preserves (76.1% of its total amount in the diet), including potatoes (17.1%), cabbage (15.5%) and beets (13.7%) [40]. For comparison, in the Belgian population, the mean intake of nitrates was 1.38 mg/kg b.w./day, corresponding to 38% of the ADI; half of the intake of nitrate/nitrite was derived from vegetables (especially lettuce) and 20% from water and water-based drinks [42].

With regard to acrylamide intake, Mojska et al. [16] reported dietary exposure to this compound in the Polish population (1–96 years) as 0.43 μg/kg b.w./day, and products such as: potato chips, French fries, cookies, sticks, corn flakes, and coffee were indicated as the main sources. Moreover, authors showed higher intake of acrylamide in children and adolescents [16]. The JECFA reports have shown that children had 2–3 times higher intake of acrylamide compared to adults caused by higher consumption of starchy foods such as cereal and baked products [33]. Zając et al. [43] also showed that total dietary exposure decreased with age, from 1.51 μg/kg b.w./day acrylamide for the youngest group (6–12 years old) to 0.67 μg/kg b.w./day acrylamide for the oldest (42–60 years old), indicating changes to diet across ages.

According to JECFA reports, the overall acrylamide intake shows a potential to reduce as a result of decreased acrylamide content in food but also changing dietary habits in societies [35]. This trend is also observed in the Polish population. The authors' research showed average acrylamide intake at the level of 0.23 μg/kg b.w./day, lower that values reported by Mojska et al. [16]. In general, the average acrylamide intake is comparable in European and American countries (e.g., Switzerland – 0.277 μg/kg b.w./day, Netherlands – 0.48 μg/kg b.w./day, Canada – 0.288 μg/kg b.w./day), and the main sources of this compound are: potato crisps, fried potatoes, bread and breakfast cereals, coffee and biscuits [33]. It should be taken into consideration that total acrylamide intake in the authors' study did not account for the contribution of acrylamide from cigarette smoking. In fact, the authors observed that smokers were exposed to almost double the amount of acrylamide (0.4 vs. 0.21 μg/kg b.w./day, without statistical significance), which may indicate their less healthy lifestyle.

Interestingly, the authors expected that obese students would have lower DIN and higher DIA than normal-weight

students, but the authors did not observe any significant differences in subgroups divided by BMI value. In the authors' study, obese subjects consumed more nitrates than the Me value for the whole group, which may have a positive therapeutic effect in the view of its postulated role in increasing adipocyte mitochondrial respiration and dampening inflammation and OS [44]. Moreover, obese participants had the lowest acrylamide intake (Table 1), which may indicate their proper eating habits, not related to increased consumption of acrylamide-rich food at the time of the study. A few links between obesity and acrylamide intake can be found in scientific reports. Lee et al. [45] indicated to acrylamide-induced adipogenesis in cell cultures. Authors reported increased expression of adipocyte fatty acid-binding protein, lipoprotein lipase, sterol regulatory element-binding protein-1c, and fatty acid synthase in murine preadipocytes treated by acrylamide solution. Also, several human studies have shown a link between dietary exposure to acrylamide and various metabolic-related outcomes. Wan et al. [46] reported the association between acrylamide-hemoglobin adducts, which are considered as biomarkers of acrylamide exposure, and the prevalence of metabolic syndrome among American adults. There is also evidence that exposure to elevated levels of acrylamide during the prenatal period resulted in increased prevalence of overweight in early childhood [47]. However, it should be noted that acrylamide exposure may be one of many contributing factors to obesity, and more research is needed to confirm this association.

While there are many links between nitrogen-containing xenobiotics and OS in literature data, and much research on their effect on different biomarkers can be found, the results in human studies are not consistent and need to be verified. Specifically, it has not been defined what levels of dietary exposure to nitrates and acrylamide can be deemed safe as the assessment of exposure is still uncertain [9,48]. To achieve the intended goal, the authors assessed selected parameters related to OS. The authors' panel included 12 markers, both enzymatic and non-enzymatic. Analyzing values obtained for the study population, the authors did not observe any spectacular changes between the lowest and highest consumption of nitrogen-containing xenobiotics and any apparent trend in subgroups of students divided by the level of their exposure. Only disulfide level increased significantly (by 63%) in the subgroup with the highest DIN compared to the lowest, and this was probably extrapolated into increase in total thiol level (by 14%). This is consistent with a study presented by Norouzirad et al. [49], in which the authors showed a lack of statistically significant differences in TOS, TAC, OSI, catalase, superoxide dismutase and glutathione (GSH) level between control and nitrate-treated rats.

Simultaneously, the authors observed that diabetic rats exposed to dietary nitrate are more prone to OS manifestation, which may lead to the interesting conclusion that a healthy organism should easily neutralize the adverse metabolic changes occurring under increased nitrate exposure [49]. Single animal studies assessed decreased GPx and GR after administration of nitrates, but no significant changes were observed in enzymatic parameters in the authors' study group [50]. Inversely, Tian et al. [51] reported that dietary nitrate attenuated OS through inhibition of NADPH oxidase, and hypothesized that they may fuel a nitrate-nitrite-NO pathway with a beneficial effect on the vascular endothelium.

In the case of DIA, the authors observed gradual decline in GPx concentration (18% difference between first and fourth quartile, $p < 0.05$). Upregulation of GPx, an important antioxidant enzyme, may indicate attenuated antioxidative response in subjects with the highest consumption of acrylamide [52]. Ghamdi et al. [53] also showed a significant decrease in the concentration of GPx in healthy men after acrylamide ingestion, but authors also revealed GR depletion, which was not observed in the authors' study group [53]. However, the authors also noticed an

unexpected increase in native thiol level with simultaneous unchanged GST concentration, which is difficult to explain and needs to be verified by future research. Maybe some other compensative mechanism, not investigated in this study, was launched, or else the result is random. Markovic et al. [51], who showed no effect of acrylamide exposure on protein thiol groups, hypothesized that it can be potentially explained by the binding of substrates containing disulfide bonds to thioredoxin that support the maintenance of unoxidizedcytosolic cysteine [54,55]. According to Zhao et al. [53] GSH depletion is indicated as the final consequence of increased acrylamide exposure, since glycidamide can be conjugated to GSH as a detoxifying pathway of acrylamide. In general, most available literature data indicate OS induction by acrylamide, which is characterized by intensive lipid peroxidation (reflected by 8-iso-prostaglandin-F2α elevation), oxidative DNA damage (8-hydroxy-deoxyguanosine elevation), increased levels of protein-carbonyl as well as a decreased intensity of enzymatic and non-enzymatic antioxidant processes [56]. Apart from the abovementioned effects, the authors also observed a higher TOS level in men, who has also higher DIA, which fits into this scheme.

Moreover, some differences in DIN and DIA observed in the study population divided by sex should be briefly commented. The differences in intake of nitrogen-containing xenobiotics with the diet, depending on sex, have been already reported in literature data [57]. Generally, women show a tendency to make healthier food choices and are much more concerned about the importance of food choices and eating behavior than men. This attitude is also reflected by dietary profiles in terms food intake patterns, which show consistent trends according to gender [58]. Additionally, glutathione reductase, TOS, uric acid and albumin was significantly higher in men compared to women, which may be associated with specific endocrine regulation as well as different eating habits (lower intake of nitrates and higher of acrylamide by men) [59,60]. Hansen et al. [61] confirmed in an animal model that nitrate acts as an endocrine disrupter, affecting hormonal balance and hormone production, especially in the prenatal period. Acrylamide may also affect the sex hormone system, especially in the prepubertal period [62].

Literature data indicates that the impact of nitrogen-containing compounds delivered with the diet on human health is poorly understood, and it is impossible to say what level of dietary exposure to nitrates and acrylamide can be deemed safe as the assessment of exposure is still uncertain [9,48]. Moreover, there is a lack of information and examinations about its impact on the parameters of redox state and its relation with parameters of oxidative-antioxidative balance.

CONCLUSIONS

Neither nitrate nor acrylamide intake markedly influenced redox state parameters in healthy young adults. However, some changes were revealed in particular parameters, especially between subgroups with the lowest and highest xenobiotic intake, reflected by DIN and DIA. Specifically, disulfide level increased significantly in the subgroup of the highest DIN, which probably resulted in the increase in total thiol level in this group. The most significant differences between subgroups with the lowest and the highest DIA were revealed for GPx and native thiol. However, the authors believe that too much importance should not be attached to this observation. It can rather be concluded that, in healthy young adults, compensative mechanisms maintaining proper redox status are efficient enough to neutralize OS induced by increased exposure to nitrogen-containing xenobiotics delivered with food, as long as it remains within an acceptable range.

Limitations of the study

There are a few noteworthy limitations to this study. The nitrate and acrylamide intakes were assessed on the basis of dietary habit survey, while it would be more accurate to

measure the amount of these compounds in the reconstituted diet or to determine biomarkers of exposure in blood and urine. However, in the study the authors used a sFFQ method, the reliability of which was confirmed by the high correlation coefficient with results from the 7-day recall performed in part of the study participants. Moreover, the sFFQ method is easy to perform and non-invasive. The authors are also aware that the size of the group was not large, but the results obtained are interesting therefore it is imperative to continue research in this area.

Author contributions

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