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A Preliminary Study Investigating the Effects of Elevated Antioxidant Capacity of Daily Snacks on the Body's Antioxidant Defences in Patients with CVD

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Featured Application: The adequate dietary antioxidant capacity levels determined in this study can be used in planning prevention and diet therapy for patients with CVD.

Abstract: The antioxidant potential of foods plays a vital role in counteracting oxidative stress and its consequences in the body. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the primary line of defence against cellular damage caused by reactive oxygen species (ROS). Glutathione is considered to be the most vital antioxidant for the body because its changes during oxidative stress increase the risk of CVD. The dietary antioxidant capacity supporting the glutathione defence system is not known. Therefore, we analysed the glutathione defence-related markers changes in the serum of CVD patients under the dietary supplementation of increased antioxidant capacity snacks. Patients were split into groups according to inclusion criteria and dietary intervention (DI) design. The serum concentration of GPx and GST (glutathione-S-transferase) was measured before and after the 6-week DI. During the DI, CVD and control (CON) subjects increased the total diet antioxidant capacity by 48% and 21%, respectively. It resulted in a significantly decreased GST (from 3.71 to 2.54 U/g Hb, $p < 0.05$) and an increased GPx (from 33.90 to 38.3 U/L). The results in the CON group did not reveal significant changes in GST and GPx. This study demonstrated that an increased antioxidant capacity might be associated with improving glutathione-related defence. However, the conclusion is not substantial due to the small sample used in this study.

Keywords: antioxidants; glutathione peroxidase; glutathione-S-transferase; dietary intervention; cardiovascular disease; antioxidant capacity; ORAC

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1. Introduction

Cardiovascular disease (CVD) is the most significant global non-communicable disease (NCD), which is responsible for a third of total mortality [1]. The search for an effective antidote and prevention of this disease's mechanism is still in progress [2,3]. Many risk factors including lifestyle modifications, drug use, tobacco smoking, and dietary considerations have been defined [4]. Accumulating scientific evidence suggests that oxidative stress (OS) is also a common pathway for developing CVD. Concurrently in the last decade, the diet antioxidant capacity as a factor in preventing oxidative stress mechanisms has been raised in scientific discussions. It has been shown that patients at risk of CVD have enhanced OS and could benefit from dietary antioxidant treatment [5]. Although the benefits associated with antioxidant-rich foods intake are known, the precise doses capable of lowering oxidative stress or improving the body's antioxidant potential have not yet been determined.

Some research revealed relationships between OS and dietary antioxidant intake [6–12]. It has been shown that OS markers improved after serving fresh fruits, vegetables, chocolate, and red wine [7,8,10,13]. Other results revealed that consuming 160 g of potato chips daily increased oxidative stress and inflammatory markers [11]. Although these studies showed the possibility of regulating the body's antioxidant capacity, their results do not allow for a straightforward and practical interpretation of dietary recommendations for CVD patients. It is worth noting that studies on well-monitored organism antioxidant capacity changes related to a dietary supply of natural antioxidants are missing.

To understand how dietary intervention (DI) increases the body's antioxidant capacity, it is essential to note that firstly, the antioxidant capacity of the dietary intake should be monitored; and secondly, biomarkers of OS should be fitted precisely and analysed. The dietary antioxidant capacity is analysed in the literature with many markers. Since antioxidants are a chemically diverse group of compounds, one popular compound is the oxygen radical absorbance capacity (ORAC) [14–16]. The biomarkers of OS (C-reactive protein, interleukin-6, tumour necrosis factor-α, and F2 isoprostane) and its reactions to DI have also been studied in the literature [6–12]. Nevertheless, their reproducibility and sensitivity regarding dietary intervention varied, especially when a practical approach to dietary recommendation was needed.

It is worth noting here that changing the dietary patterns leading to permanent modifications in dietary intake is challenging for many patients and thus should be related to simple and realistic recommendations [17]. Therefore, the focus should be on minor nutritional modifications based on existing eating habits, such as snacking [18,19].

Therefore, when undertaking DI we decided to use red beetroot and chokeberry snacks, which are popular plants with known health-promoting properties recognised by respondents in Poland.

Another issue is monitoring diet-induced changes in the body's antioxidant capacity. Scientific debates on OS markers and the organism's antioxidant capacity were presented in the literature [17,20–22]. It was concluded that antioxidant capacity biomarkers are better antioxidant defence forecast in pathophysiological conditions than OS markers [23–25]. Under physiological conditions, an antioxidant system defends the cells from ROS-induced damage [26]. The most important antioxidant enzymes are SOD, catalase (CAT), and GPx [27]. These enzymes represent the primary line of ROS defence, and glutathione, among them, is perceived as the most vital antioxidant for the body. Therefore, it is pertinent to observe the glutathione defence-related markers.

Glutathione (GSH) is a tripeptide, modulating cell responses to redox changes, detoxifying the drug's metabolites, regulating gene expression and apoptosis, and is involved in the transmembrane transport of organic solutes [28]. GSH might be easily oxidised and regenerated very rapidly. This allows it to be the primary intracellular antioxidant, a modulator of cell proliferation and immune responses and to help regulate signal transduction within cells. The GSH buffer system modulates cell response to redox changes. Reactive oxygen species (ROS) and reactive nitrogen species (RSN) are reduced or inactived through the generation of a disulfur bond between two glutathione molecules to form oxidised glutathione [29–31]. Concerning xenobiotics-inducing cancers, glutathione makes epoxides less toxic. In dietary oxidant actions generating ROS, GSH reduces or inactivates ROS by generating disulfur bonds between two glutathione molecules to oxidise glutathione [30]. GPx is the general name of an enzyme family with peroxidase activity. It has a biological role in protecting the organism from oxidative damage. The biochemical function of GPX is to reduce lipid hydroperoxides in their corresponding alcohols and to reduce free hydrogen peroxide in water [29,32]. Glutathione S-transferases (GSTs) are enzymes responsible for detoxifying reactive chemical species through conjugation with reduced glutathione. GSTs are essential mediators in OS responses, are involved in synthesising prostaglandins, and facilitate the intracellular transport of hydrophobic compounds [30].

Despite the apparent benefits of DI on health outcomes, the independent effect of diet antioxidant capacity during DI on the glutathione-related antioxidant defence system

remains unclear. The effective dose of antioxidant capacity guaranteeing the improvement of the GPx and GST markers was not identified.

These facts prompted us to look for healthy and high antioxidant capacity snacks, including daily snacks that could improve the antioxidant capacity monitored by GPx and GST enzymes. The availability of such snacks and their antioxidant value were discussed and presented in previous studies [31,33–35]. Based on it, we decided to use beetroot crisps and chokeberry juice.

With this in mind, we aimed to analyse glutathione antioxidant defence system changes in CVD patients to establish the healthy snacks needed to enhance the body's antioxidant capacity.

2. Materials and Methods

2.1. Study Sample

Twenty-nine unrelated men aged 40–75 years were recruited via advertising at local hospitals in the Poznan area; the men voluntarily participated in this study. The mean BMI was 28.2 and 27.7 kg/m^2 in the CVD and CON groups, respectively (Table 1). Initially, both women and men were selected for this study. However, men were chosen as the final sample due to their higher risk for CVD, a higher drop-out rate among women (63%), and because of many other co-morbidities preventing participation of other people. The diagnosis of the selected participants was confirmed at the Clinical hospital.

Table 1. Characteristics of the study sample.

Variables	CVD	CON
n	19	10
Age (years)	58 ± 12 a	56 ± 8.6 b
Weight ^{0} (kg)	86.8 ± 12.8	79.0 ± 14.8
Weight ^{1} (kg)	86.1 ± 12.3	79.0 ± 14.8
Weight ^{2} (kg)	86.4 ± 12.0	79.4 ± 14.5
BMI ^{0} (kg/m^2)	28.2 ± 4.0	27.70 ± 3.9
BMI ^{1} (kg/m^2)	28.0 ± 4.0	27.80 ± 4.8
BMI ^{2} (kg/m^2)	28.1 ± 4.0	27.80 ± 4.8
WHR ^{0} (−)	0.96 ± 0.06	0.96 ± 0.07
WHR ^{1} (−)	0.96 ± 0.05	0.96 ± 0.07
WHR3 ^{2} (−)	0.97 ± 0.05	0.96 ± 0.05

The inscription letters inform about the statistical differences between the stages of the research of both groups separately. a, b statistically different values are marked with varying inscriptions of letter ^{0} The measurement before the 1st phase of the DI. ^{1} The measurement at the beginning of the 2nd phase, the intervention period. ^{2} The measurement at the end of the 2nd phase.

The CON group was potentially healthy subjects chosen by the CVD patients. Participants qualified for the CVD group were asked to indicate or recommend healthy colleagues, who were of similar age and were characterised by an equal socioeconomic status and education levels.

The following were the inclusion criteria: Acute coronary syndrome in the last 24 months, age between 45 and 75 years, written consent for anticipation in the study, no contradictions from the attending physician, diagnosis of CVD thesis confirmed by coronarography, stable (lack of changes) pharmacological treatment during the DI, and lack of diet modification (e.g., Ketogenic, low-calorie, and low-histamine). Exclusion criteria were smoking, chronic diseases (tumours, tuberculosis, diabetes type I, and heavy physical exercise (10 h/week), and vitamin or mineral-supplements intake.

An internal medicine doctor interviewed all patients before the intervention. During the interview, the occurrence of acute coronary syndrome in the last 2 years was confirmed. Medications used were verified and controlled during the intervention. Their doses and types were constant and did not change. The patient's condition was described as stable. The subjects received verbal and written information about the study before giving their written consent. All subjects completed the study.

The Scientific Ethics Committee approved the study at the Poznan University of Medical Sciences (Resolution No. 584/11). The project followed the ethical standards recognised by the Helsinki Declaration.

2.2. Study Design

The study design is presented in Figure 1.

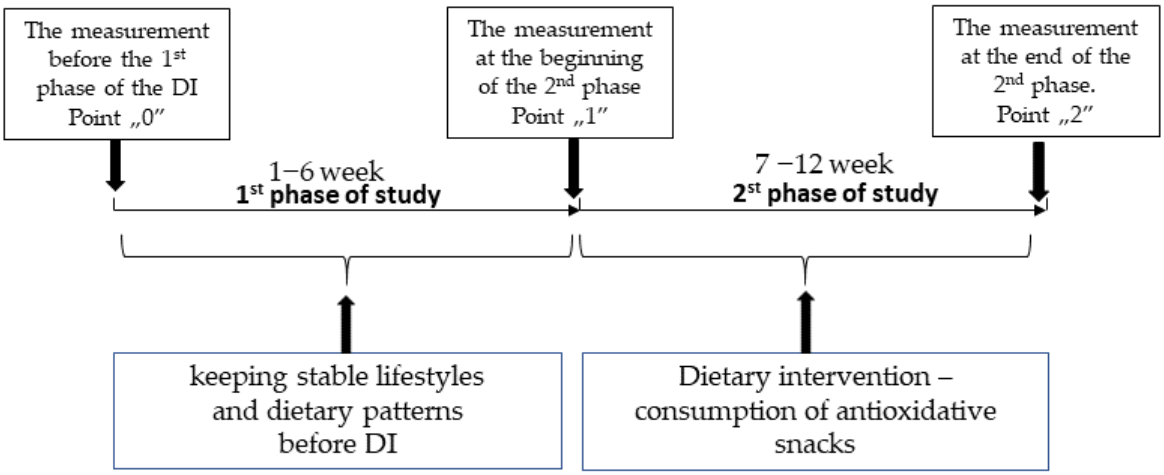

Figure 1. Study design.

The study was conducted as a controlled trial. Subjects supplemented their usual diet with one of the two snacks daily. The first was a package of 15 g of beetroot crisps, prepared especially for DI via microwave drying [36]. The second was a commercially available juice bottle (330 mL), the chokeberry (Aronia) juice. The nutritional value of snacks is presented in Table 2, and the total intake was assessed based on both groups' dietary records.

Table 2. The nutritional value and antioxidant capacity of snacks used in DI.

Variables	Beetroot Crisps [1] 100 g	Beetroot Crisps Package 15 g	Chokeberry [2] Juice 100 mL	Chokeberry Juice 330 mL
Energy (kcal)	396	59	50	165
Proteins (in total) (g)	11.7	1.7	0.00	0.00
Total Carbohydrates (g)	79.2	11.9	12.5	41
Total fat (g)	0.6	0.1	0.01	0.00
Calcium (mg)	402	60.3	17.5	57.8
Iron (mg)	1.3	0.19	1.5	5.0
ORAC (μMTx)	201,416	17,250	771,598	66,083

[1] presently commercially available snacks, data identified during the project implementation [37]; [2] analysed and published previously [33,38].

The study period was divided into 2 phases (12 weeks). In the first phase (6 weeks), subjects were asked to keep their usual lifestyle, diet and pharmacotherapy. Before and directly after the first period, blood samples were collected, and nutritional status (anthropometry and body composition) was analysed. In the second phase, i.e., the intervention period (next 7–12 weeks), participants were instructed to eat all the provided snacks in random order once a day and to keep daily records of the consumed snacks and keep their packaging. After the DI, blood samples were collected for the third time, and the intake of all snacks was calculated. Surprisingly, the consumption of both snacks during DI was comparable (50:50).

2.3. Dietary Data Collection

In both phases, an estimated food record method was applied, covering seven consecutive days, five weekdays (Monday–Friday) and two weekend days (Saturday–Sunday) [39]. The procedure was described in our previous studies [40–44]. Respondents recorded the intake of all foods and beverages in paper food dairies continuously throughout the day. During the second visit, respondents were instructed to write down the type and the brand name of the product, along with the weight (displayed on the product label) and time consumed. Alternatively, if the product was not entirely consumed or its weight was unknown, the respondents were asked to write down portion size in household measures (e.g., small cup, little bowl and large plate). If the food was homemade, respondents were asked to record the type, brand name, and weight of all ingredients, along with a description of food preparation, cooking method (e.g., cooking, frying and grilling), and cooking time. If eating out, respondents were asked to record the type of food, portion size, and restaurant name (if part of a chain). Researchers verified all the food records and completed questionnaires during interviews with the respondents (third visit). The amount of food was determined using an "album of photographs of food products and dishes" [45] and expressed in grams. The mean daily energy and selected nutrient intake were calculated using "Energia v.4.1" software with an implemented food database. The food database was composed of 1178 products: 962 from the official database of foods commonly consumed in Poland [46] and 216 manual inputs (from the USDA database) [47] of ethnic foods or products new on the market, which could not be found in the Polish "food composition tables" [46]. Plate waste was estimated using built-in software options and accounted for 10% of energy and all the macro- and micronutrients (10%), except for vitamin C (55%), folic acid (40%), vitamin A (25%), B1 (20%). B2 (15%), and niacin (15%). Diet antioxidant capacity was also calculated.

Total dietary antioxidant (ORAC) intake was calculated from the food records using the USDA ORAC database of selected foods [48]. In particular, total dietary antioxidant intake was calculated according to the methodology published already in our previous studies [49]. The average intake of each food item was calculated and subsequently multiplied by the respective total ORAC value of the database [49]. Total dietary antioxidant intake and oxygen radical absorbance capacity values were expressed as μmol Trolox equivalents (TE)/day.

Snacks were delivered to the subjects weekly, and records were collected. Consumption of one snack daily for the 6 weeks of DI was required. Subjects could randomly choose snacks and were asked to mark their choice and the amount eaten on the protocol. Packagings after consumption and protocols were collected weekly. The number of snacks eaten daily was calculated.

2.4. Blood Samples

Blood sampling and laboratory methods venous blood samples were collected from the subjects at 08:00 h after a 12 h overnight fast as described previously [50]. Three collections were planned: at the study's beginning (point 0: before 1st phase of DI), after 6 weeks, 1st phase (point 1: after keeping a stable lifestyle and dietary patterns before DI), and after and at the end of DI (point 2: after the 6 weeks of DI with high antioxidant capacity snacks). Samples were centrifuged at 3000 rpm for 10 min at 4 °C within 2 h after blood collection and were stored at −80 °C for further analysis.

GST activity and GPx activity were measured in duplicate, using the available kits, according to the manufacturer's (Randox, Laboratories Ltd., Warsaw, Poland) protocol [45].

2.5. Statistical Analyses

Post hoc Power Calculator (ClinCalc, LLC) was used to calculate the study power [51]. The calculation was based on means and standard deviations of GST. Assuming a two-sided significance level of 0.05, the study power was found to be 79.9%.

All variables were checked for normality with the Kolmogorov–Smirnov test. Data are presented as means ± SD. Physical baseline characteristics of participants were compared between CVD and CON using the *t*-test and Mann–Whitney test for independent samples. The effects of DI (before and after the 6-week antioxidant snacking period) were estimated using the Wilcoxon test. Statistical analysis was carried out using TIBCO Software Inc. (2017), Statistica (data analysis software system), version 13. The significance level was set as $p < 0.05$.

3. Results

All samples during the experiment were collected in three points (Figure 1). Point "0"—the 1st phase for qualification; point "1"—the wash-out period (1st phase) before including DI snacks; and point "2"—the end of DI with antioxidative snacks (2nd phase).

The physical baseline characteristics of participants were compared between CVD and CON groups (Table 1). No differences in BMI and WHR between CVD and CON groups were observed during the qualification phase. There were no changes in BMI and WHR between the experiment's 0th, 1st, and 2nd points within each group.

Table 3 shows both groups' glutathione-related antioxidant capacity of plasma changes. GST and GPx were significantly ($p < 0.01$) higher in the CVD than in the CON group before the 1st phase of the experiment (Point "0"). After qualification to the study, participants of both groups were asked to keep stable lifestyles, medication, physical activity and dietary patterns for 6 weeks before DI. This experiment phase was planned as the wash-out period (the pre-determined period).

Table 3. The glutathione-related antioxidant capacity changes in the study sample during 6 weeks of DI.

Variables	CVD (n = 19)	CON (n = 10)
GST [0] (U/g Hb)	3.43 ± 1.75 a	2.37 ± 0.65 b
GST [1] (U/g Hb)	3.71 ± 1.44 a	2.37 ± 0.65 b
GST [2] (U/g Hb)	2.54 ± 0.81 b	2.56 ± 0.78 b
GPx [0] (U/L)	31.85 ± 9.80 a	27.38 ± 6.56 b
GPx [1] (U/L)	33.90 ± 5.85 a	27.38 ± 6.56 b
GPx [2] (U/L)	38.3 ± 10.19 b	26.86 ± 7.12 b

The inscription letters inform about the statistical differences between the stages of the research of both groups separately. a, b statistically different values are marked with different letter inscriptions. [0] The measurement before the 1st phase of the DI. [1] The measurement at the beginning of the 2nd phase, the intervention period. [2] The measurement at the end of the 2nd phase.

There were no differences in GST and GPx concentration between points "0" and "1" in both CVD and CON. After DI in point "2", the CVD group indicated a significant ($p < 0.05$) decrease in GST from 3.71 to 2.54 U/g Hb and an increase ($p < 0.05$) in GPx concentration from 33.9 to 38.3 U/L. In the CON group, we did not observe significant changes in both GST and GPx.

The value of snacks designed for consumption in DI is presented in Table 3. According to the subject's records, snacks were ingested randomly between days. Subjects were obligated to record the snack consumption for each day (amount and quality).

Conferring to the subjects' information, its ingestion for 6 weeks increased diet antioxidant capacity by 924 and 1167 μMTx/daily in the CVD and CON groups, respectively. This increased total diet antioxidant capacity by 48 and 24% in CVD and CON groups, respectively.

There were no differences between 1st and 2nd phases of DI in dietary intake; therefore, data from the 1st phase were not included in analyses. Table 4 shows the diet's nutritional value during the 2nd phase of DI in the CVD and CON groups. The comparisons of nutritional value the dietary intake showed differences between groups. Men in the control group had a significantly higher antioxidant capacity ($p < 0.01$), lower total proteins ($p < 0.05$), total fats $p < 0.05$, SFA ($p < 0.01$), MUFA ($p < 0.01$), cholesterol ($p < 0.01$), sodium

($p < 0.01$), potassium ($p < 0.01$), retinol ($p < 0.01$), vitamin D ($p < 0.01$), vitamin E ($p < 0.001$) niacin ($p < 0.05$), folates ($p < 0.05$), and vitamin C ($p < 0.01$).

Table 4. Dietary intake of the study sample during 2nd phase of the DI and CON group.

Variables	CVD	CON
ORAC (μMTx/day)	1924 ± 654	4865 ± 1865 **
$ORAC_{snacks}$ (μMTx/day)	924 ± 154	973 ± 92
Energy (kcal)	2420 ± 355	2087 ± 681 *
Proteins (in total) (g)	97 ± 47	64.5 ± 19.4 *
Total fats (g)	111 ± 39	79.1 ± 29.2 *
% energy from proteins	12.8 ± 1.4	10.6 ± 1.6
% energy from fats	36.9 ± 8.7	26.0 ± 3.4
% energy from carbohydrates	50.2 ± 8.7	49.7 ± 4.0
Saturated fatty acids SFA (g)	35.6 ± 29.8	28.7 ± 13.2 **
Monounsaturated fatty acids MUFA (g)	45.5 ± 29.5	27.1 ± 12.7 **
Polyunsaturated fatty acids PUFAS (g)	22.2 ± 16	17.1 ± 7
Cholesterol (mg)	359 ± 187	220 ± 76 **
Total carbohydrates (g)	315 ± 142	302 ± 93
Fibre (g)	28.2 ± 11	23.6 ± 7.8
Sodium (mg)	3159 ± 810	1689 ± 169 **
Potassium (mg)	54,951 ± 333	3738 ± 1484 **
Calcium (mg)	567 ± 188	601 ± 342
Phosphorus (mg)	1386 ± 509	1246 ± 382
Magnesium (mg)	368.6 ± 131	337 ± 96
Iron (mg)	14.1 ± 6.0	11.7 ± 3.0
Zinc (mg)	12.9 ± 5.5	10.1 ± 2.3
Vitamin A (retinol equivalent) (μg)	1488 ± 651	896 ± 300 **
Vitamin D (μg)	5.4 ± 2.5	2.5 ± 0.7
Vitamin E (alpha-tocopherol equivalent) (μg)	18.5 ± 5.8	2.5 ± 0.7
Thiamine B1 (mg)	1.5 ± 0.8	1.2 ± 0.6
Riboflavin B2 (mg)	1.5 ± 0.6	1.4 ± 0.5
Niacin B3 (mg)	19.1 ± 5.9	14.7 ± 6.4
Pyridoxine B6 (mg)	2.5 ± 0.9	2.1 ± 0.9
Folates (μg)	369 ± 149	191 ± 49.5
Cyanocobalamin B12 (μg)	4.3 ± 2.6	3.3 ± 1.5
Vitamin C (mg)	149.1 ± 34.6	81.7 ± 70.2

* $p < 0.05$; ** $p < 0.01$.

4. Discussion

The present study is an original investigation to verify the effect of antioxidant snacks intake and a regular diet on the glutathione-related antioxidant defence system expressed by GPx and GST. To our best knowledge, this is the first study to apply a customised DI in CVD patients and evaluate its effectiveness in promoting their nutritional habits, particularly healthy snacking, to increase diet antioxidant capacity. Following the aim of this project, we established that high-antioxidant snacks, such as juice or vegetable crisps, might enhance the body's antioxidant capacity. We observed significant ($p < 0.01$) improvement in the GPx and decrease in the GST (0.05) following changes in daily dietary snacking with either a 15 g package of beetroot crisps or a 330 mL bottle of chokeberry juice. This modification increased the participant's diet antioxidant capacity by 48% (CVD) and 24% (CON) in relation to their habitual consumption and resulted in a 32% decrease in GST and a 13% increase in GPx blood concentration.

First, it should be noted that despite the increase in the diet antioxidant capacity in both groups, changes were observed only in the group with CVD. This group presented significantly higher levels of GST ($p < 0.01$) and GPx ($p < 0.01$) at the beginning of the intervention. In addition, the diet analysis in both groups also showed that the CVD subjects had a higher energy density related to the supply of saturated fat, protein, cholesterol and sodium, significantly reducing the body's antioxidant capacity. This poor diet quality was

confirmed by assessing their antioxidant capacity without antioxidant snacks. These factors may have contributed to the higher initial levels of oxidative stress in CVD patients and caused them to respond to the inclusion of antioxidant snacks in their diet. Such changes were not observed in the CON group; however, the diet's nutritional value was much higher, and the level of markers was lower than in CVD subjects.

While GST and GPx are involved in maintaining cellular homeostasis and protecting against harmful substances, their specific enzymatic activities and substrate specificities differ. GST primarily focuses on detoxification pathways, while GPx is primarily responsible for protecting cells from oxidative damage. The GSTs are structurally highly diverse enzymes protecting against reactive α,β-unsaturated carbonyls, epoxides, and hydroperoxides produced in vivo as the breakdown products of macromolecules during oxidative stress [52–54]. Changes in GST levels induced by a diet high in glucosinolates have already been observed by other authors, who noted an increase in GST levels after a 3-week consumption of 300g of cooked Brussels sprouts per day [55,56]. In our study, changes in GST concentrations were induced by betalains and anthocyanins present in red beetroot and chokeberry.

In contrast, the lack of change in GST concentration in the control group, in which oxidative stress levels were probably lower and the diet better balanced, seems interesting but explainable. We suggest that changes in the glutathione-related antioxidant defence system will be related not only to the dose and type of dietary antioxidants consumed in the diet but also to the initial antioxidant capacity of the body.

Additionally, of interest, in terms of the outcome of the dietary intervention, were the changes in glutathione peroxidase (GPx) levels induced by the same dietary intervention. We observed a significant increase ($p < 0.01$) of GPx in the CVD group, but no changes in the CON group. GPx catalyses the reduction of H_20_2 and organic hydroperoxides to water and alcohol by generating GSSG (glutathione disulfide). Several distinct families of enzymes have evolved that display GPx activity. These have been classified as selenium-dependent or selenium-independent [57]. Our results present GPx concentration before DI can be compared with those existing in the literature [58]. Additionally, an increase in GPx activity under the influence of nutritional interventions with Brazilian nuts corresponded to those obtained in our experiment during snacking with chokeberry juice and beetroot crisp [58]. The Brazilian authors explained that th increased GPx concentration was due to the increased erythrocyte selenium levels [58]. In our study, we did not identify any rich source of selenium.

In our study, we selected two snacks recognised as healthy and recommended by the CVD Polish patients. We confirmed this popularity and willingness to snack during the qualification stage. These snacks are commonly consumed by individuals with cardiovascular disease (CVD). Although the beetroot crisps had a lower antioxidant capacity than aronia juice, they were more widely favoured as a vegetable among the Polish population. Though the popularity and presence of aronia juice on the market is apparent, its sensory acceptability is meaningfully lower because of its tart taste.

Additionally, the antioxidative properties of both snacks differed and were related to different compound classes. The beetroot crisps mainly contain high content of betalains, while the chokeberry juice contains mainly flavonoids. Since we planned to evaluate the general effect of diet supplementation and increased general diet antioxidant capacity on the pilot level of study, betalains and flavonoids were chosen as both classes have a beneficial effect on the cardiovascular system [59–61].

Dietary antioxidant capacity is perceived as a new pro-healthy indicator of diet quality and has been studied previously in CVD and prediabetes group [62,63]. Those studies showed that higher dietary antioxidant capacity was associated with glutathione-related antioxidant defence system changes. Likewise, in Greek studies that included daily consumption of red wine, authors revealed twice the increase in antioxidant capacity and reduced cardiovascular risk [10]. Our data support the previous studies reporting beneficial influences of increased diet antioxidant capacity on health outcomes [13,64–67]. An

interesting finding was the differences observed in glutathione defence system reaction in CVD and CON groups.

When discussing the beneficial effect of antioxidants in maintaining the antioxidant-pro-oxidative balance, it should be noted that there have been reports and discussions of their pro-oxidative effect in the literature [68,69]. Such data in the literature may cause concern regarding the widely promoted consumption of antioxidants. For example, the CARET intervention was stopped 21 months early because of clear evidence of no benefit and substantial evidence of possible harm (28% more lung cancers and 17% more deaths in the active intervention group, active = the daily combination of 30 mg beta-carotene and 25,000 IU retinyl palmitate) [70]. However, before we draw far-reaching conclusions, we must analyse the factors, and the group and form of administration of antioxidants. The allegations against antioxidants almost have always concerned dietary supplements and not their natural supply in the diet. The doses planned in the experiment exceeded the physiological requirements specified in the dietary standards (Carotene and vitamin E). The supplements were administered to cancer-risk groups (e.g., smokers and those working with asbestos). Finally, the hypothesised influence was relayed in vitro studies where ROS increase induced ROS defence and improved healthspan in the long term [69]. To conclude, it is necessary to underline that these assumptions remain speculative. The best advice would be to ingest antioxidants from food sources rather than from self-prescribed supplements.

The results of this intervention study imply that snacking high antioxidant capacity refreshments might significantly improve the glutathione-related antioxidant defence system. We observed a 32% decrease in GST activity and a 13% increase GPx activity.

However, this research may have some limitations. Particularly, the limited number of subjects is a major drawback. In addition, one should also be aware that snacking is often considered an inappropriate eating habit that many people actively try to reduce, which may have influenced the amount of snacks they eat. Even though respondents voluntarily accepted to consume the snacks, this should be kept in mind.

It should be emphasised that the obtained results could be supported by other antioxidant capacity markers, such as SOD, which are the missing elements in the whole antioxidant defence and could provide a complete picture of the antioxidant defence in CVD patients.

5. Conclusions

This study demonstrated that an increased diet antioxidant capacity was significantly associated with improving glutathione-related defence. Our data strengthen the concept that promoting antioxidant dietary recommendations for preventing and treating cardiovascular diseases should be considered. To further explore the potential benefits of dietary changes in combating cardiovascular disease, prospective studies should consider using ORAC as a marker of diet antioxidant capacity. Measuring GPx and GST concentrations in studies to estimate changes and potential benefits of nutrition in patients with cardiovascular disease would be advisable.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Poznan University of Medical Sciences, Resolution no. 584/11. Informed consent was provided and signed by participants.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

BMI	body mass index
CAT	catalase
CVD	cardiovascular disease
DI	dietary intervention
GPx	glutathione peroxidase
GST	glutathione S-transferases
ORAC	Oxygen Radical Absorbance Capacity
OS	oxidative stress
ROS	reactive oxygen species
SOD	superoxide dismutase
WHO	World Health Organization
WHR	waist-to-hip ratio

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