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Original article

Effect of broccoli sprouts on thyroid function, haematological, biochemical, and immunological parameters in rats with thyroid imbalance

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ABSTRACT

Broccoli sprouts may exert a negative influence on thyroid function as they are a rich source of glucosinolates, in particular glucoraphanin. Under the study in a long-term experiment broccoli sprouts were tested as an element of rats diet, combined with deficient iodine, or sulfadimethoxine ingestion – two models of hypothyroidism. Evaluations were performed for serum TSH and thyroid hormones completed with analyzes of selected haematological, biochemical and immunological (IL-6, IL-10) parameters, as well as cytosolic glutathione peroxidase (GPX1), thioredoxin reductase (TR) in the thyroid, and plasma glutathione peroxidase (GPX3). A thermographic analysis was conducted to provide auxiliary indicators for determining a potential thyroid dysfunction under the specific experimental conditions. The levels of TSH, fT3 and fT4 remained unchanged following broccoli sprouts ingestion, which was even found to have a protective effect against sulfadimethoxine induced thyroid damage. Moreover, TR activity significantly increased in response to sprouts ingestion. In animals with hypothyroidism, broccoli sprouts were found to exert a beneficial influence on the antioxidant balance of the thyroid gland. In comparison to the rats with iodine deficiency, broccoli sprouts addition to the diet was observed to decrease IL-6 level. No significant differences in IL-10 concentration were determined.

Neither addition of broccoli sprouts to the diet, nor sulfadimethoxine and iodine deficiency, caused negative changes in red blood cell parameters, glucose and uric acid concentrations, or kidney function. However, such a dietary intervention resulted in reduced WBC and PLT levels, and it may adversely interfere with liver function in rats, most likely due to a higher dietary intake of glucosinolates.

1. Introduction

Broccoli sprouts have been long recognized as one of the most significant and well known elements of functional food. The sprouts have been shown to exert chemoprotective effects [1,2] and to reduce cholesterol or lipid levels [3]. They are also a powerful bactericide against *Helicobacter pylori* infections [4]. They may improve insulin resistance in type II diabetes and many other positive effects have been reported for them [5]. No evidence has been found so far for broccoli sprouts to interact with thyroid function in models of potential hypothyroidism. Only Shapiro et al. [6] have published results of their clinical phase I study where safety, tolerance and metabolism of broccoli sprouts were investigated in healthy volunteers. Evaluation of the effect of broccoli on the thyroid function is interesting, as the plant is protective against thyroid carcinoma [7,8]. On the other hand, different brassica vegetables can be responsible for impairment of thyroid gland function in different species, particularly in poultry, pigs and rats [9–11]. Dal Maso et al. [8] indicated that historically reported adverse effects of very high consumption of cabbages and other cruciferous vegetables (e.g. induction of thyroid cancer) are still rooted in public opinion. The influence of such hints is observed in many dietetic recommendations regarding these vegetables presumable involvement in the induction of hypothyroidism. Despite more recent epidemiological studies exploring associations between cruciferous vegetables and thyroid diseases which give only limited support to the previous hypotheses, due to repeated public exposure to the other statements, they have become considered confirmed recommendations. Therefore, the safety of broccoli sprouts as a significant element of functional food should be revisited with respect to their role in thyroid function.

The main aim of this investigation was to study the effects of

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broccoli sprouts on the thyroid gland and on certain haematological, biochemical, and immunological parameters of a rat organism. Three diet models were applied in the experiment, namely, a normal diet, a model based on a diet with iodine deficiency causing thyroid hyperplasia [12,13], and another one based on sulfadimethoxine (SDM) added as an ingredient (0.025%) to the animal drinking water and causing thyroid damage by inhibiting thyroid hormone synthesis [14]. Serum concentration of thyroid-stimulating hormone (TSH), free thyroid hormones (triiodothyronine (fT3), and thyroxine (fT4) served as animal response parameters, while red blood cell count (RBC), haemoglobin (Hb), haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), thrombocyte count (PLT) represented haematological parameters; glucose (Glu), uric acid (UA), urea (U), aspartate transaminase (ASPAT), alanine transaminase (ALAT), creatinine (Crea), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), alkaline phosphatase (PAL) were used as biochemical parameters; and last but not least interleukin 6 (IL-6), interleukin 10 (IL-10) as immunological parameters. Enzyme activities of cytosolic glutathione peroxidase (GPX1) and thioredoxin reductase (TR) in the thyroid, ferric reducing ability of plasma (FRAP), plasma glutathione peroxidase (GPX3) activity, along with evaluated body temperature complete the list of parameters under investigation.

2. Materials and methods

2.1. Plant material

Voucher specimens of broccoli seeds (*Brassica oleraceae convar. botrytis var. cymosa*) were deposited at the Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Jagiellonian University Medical College (No#BOCB/PP/PL 1036). Four day old sprouts were harvested by the Uniflora Company, Poland. After sprouting, the materials were lyophilized to obtain dry material suitable for preparation of animal fodder. In lyophilized broccoli sprouts sulforaphane concentration was evaluated by UPLC–MS/MS method, and the mean value was 113.33 ± 12.58 mg/100 g dw. Qualitative HPLC analysis of methanol extracts of broccoli sprouts revealed a number of phenolic acids: chlorogenic, *p*-coumaric, ferulic, gentisic and sinapic acids, and also robinin and traces of myrycetin, luteolin, quercetin and apigenin. The quantitative HPLC analysis was used for predominant polyphenols: chlorogenic acid (37.26 ± 0.6 mg/100 g dw); *p*-coumaric acid (27.75 ± 0.70 mg/100 g dw); ferulic acid (73.85 ± 3.50 mg/100 g dw); gentisic acid (80.80 ± 4.79 mg/100 g dw); sinapic acid (140.53 ± 3.17 mg/100 g dw); robinin (1.04 ± 0.10 mg/100 g dw). The results of fatty acids profile showed that saturated acids in broccoli sprouts consisted 11% of total pool of fatty acids, with palmitic (5.7%) and stearic (2.8%) acids being the dominating compounds. The relative content of unsaturated fatty acids in the analyzed broccoli sprouts was found to be 89%, with predominant oleic acid (45.5%), linoleic (20.8%) and alpha-linolenic acids (17.06%).

2.2. Animals

The 72 male (mean weight 249.5 ± 9.1 g) 4-week-old Wistar rats were maintained in plastic cages in an air-conditioned animal room in the Animal House of the Faculty of Pharmacy, Jagiellonian University Medical College for one week before the experiment at the temperature of 22 ± 2 °C, with a relative humidity of 50 ± 5%, and 12 h periods of light and darkness. After 1 week of acclimatization, the rats were divided into 6 groups, each consisting of 12 animals, and fed one of the following diets: a standard diet (C); an iodine deficiency diet (DI); a diet with 7% of lyophilized broccoli sprouts (B); an iodine deficiency diet with 7% of lyophilized broccoli sprouts (BDI); a standard diet with 0.025% SDM administered to animals with drinking water (S); or a diet containing 7% of lyophilized broccoli sprouts and 0.025% SDM administered in their drinking water (BS). The rats had unlimited access to fodder and water. The diets were prepared by The Morawski Fodder Company (Poland). All the compounds present in all the diet variants (apart from C and B group) were certified as being iodine free. Detailed descriptions of the diets composition, the fodder intake, and the average amount of sulforaphane after hydrolysis are presented in Table 1. The protocols for animal experiments were approved by the Animal Experimentation Committee of Jagiellonian University, Kraków, Poland (No. 76/2014). After 8 weeks blood was collected from the abdominal aorta under thiopental anaesthesia for hormone assays and other parameters determinations, apart from haematological parameters, which were evaluated as described further. Prior to analyzes samples were stored at −80 °C.

2.3. Haematological evaluation

Blood samples of about 600 µL were obtained from rat tail veins and placed in plastic Microvette 100 K3E tubes (Sarstedt). A complete blood count was performed using an ABX COBAS MICROS Haematology automated cell counter, ROCHE. The following parameters were determined: RBC, Hb, Hct, MCV, MCH, MCHC, WBC, and PLT, presented then as 10^{12}/L, g/dL, %, fL, pg, g/dL, 10^9/L, and 10^9/L, respectively.

2.4. TSH, fT3, fT4 analysis

Thyroid hormone analyzes of serum free T4 (fT4), free T3 (fT3) and TSH levels were performed with immunoassay kits (DRG MedTek PL), according to the manufacturer's instructions. The methods have been validated for rat serum. An automatic reader (Synergy-2, BioTek/USA with syringe rapid dispensers) was used in the immunoassays. Hormone analyzes were evaluated for all rats in all groups. The concentrations of fT4, fT3 and TSH were presented as ng/dL, pg/mL and µIU/L, respectively.

2.5. Biochemical analysis

All biochemical analyzes of plasma were performed with kits (Biomerieux, France), and in accordance with the manufacturer's instructions. An ALIZE automatic biochemical analyzer (Lisabio, France) was used in the assays. Biochemical parameters were evaluated for each rat in all the groups. The concentration of Glu, U, TG, TC, HDL was presented as mmol/L, and µmol/L in the case of creatinine. ASPAT, ALAT and PAL activity were expressed as U/L.

2.6. Measurement of cytokine levels

Rat IL-6, and IL-10 ELISA kits were obtained from Diaclone (Besançon, France) and the determination of the levels of IL-6, and IL-10 were performed according to the manufacturer's instructions. The minimum detectable doses equal to 19.0 and 1.5 pg/mL, respectively, were found. Cytokine determinations were performed for 6 rats per group.

2.7. Enzyme activity and antioxidant plasma capacity analysis

The methods for determining parameters such as FRAP, GPX, TR were essentially the same as in our previous paper [15] appropriately adapted to using 48-well or 96-well plates according to Smith et al. [16]. The thyroid tissue samples were homogenized in phosphate buffer pH = 7.4. GPX3 and FRAP were evaluated in plasma. The change of absorbance during FRAP determination was measured after 8 min of incubation and the reducing ability of the sample was expressed in ferrous ion equivalents (µmol Fe^{2+}/L of plasma). TR and GPX1 were investigated in the thyroid tissue. Protein content was determined by the Bradford method (BioRad). In all of the above mentioned methods, an automatic reader (Synergy-2, BioTek/USA) with syringe rapid

dispensers was used. All the parameters indicated above were determined for all the rats in each group. For GPX1 and TR investigation in thyroid glands, analyzes were conducted in 4 rat groups, due to shortage of material. The activities of the evaluated enzymes were presented as U, mU or μU per g or mg of protein.

2.8. Thermographic investigation

The body temperature was registered at the end of experiment. A thermographic camera (ThermaCAM e300) manufactured by FLIR, with a thermographic resolution of 0.1 °C was used. Thermographic images were taken from 1 m distance in the long wave range (7.5–14 μm). Each pixel in the thermogram represents an area of 1.6 mm^2. The results of thermographic measurements have been presented as digital pictures (thermograms) which constitute a database where different temperature values are represented with different hues. Such an analysis was made between 8 am. and 11 am. under similar lighting, temperature and relative humidity conditions. Thermograms were composed with QuickReport 1.2 and Reporter 2000 Pro software. The influence of the ambient temperature and other parameters was taken into account while scrutinizing the data.

2.9. Statistical approach

All biochemical and haematological data are presented as mean values ± standard deviations. For variables with a skew distribution (marked by # in Table 2) the obtained data were transformed into logarithms and retransformed after calculations. These are presented as means with respective confidence intervals. Comparisons between groups were performed using either ANOVA with Dunnett's post hoc test, or Kruskal-Wallis test with Dunn post hoc test, for parameters that failed to meet assumptions of ANOVA test. Differences with $P < 0.05$ were considered as statistically significant. The gamma statistic was used to calculate correlations between interleukins, as some results below the detection limits were substituted by arbitral (and equal) values. The relationships between fodder consumption and hormone levels were shown only graphically, without calculating correlation coefficients, as there were two apparent lever points out of six (overall number of study groups), which must have inevitably strongly confounded possible correlation coefficients, although the relationships themselves are enlightening. The partial least square (PLS) model was used to reveal the correlation structure between the investigated parameters [17]. The set of dependent parameters consisted of fT3 and TSH while WBC, RBC, Hb, HCT, MCH, MCHC, ALAT, TG, PAL, creatinine and FRAP were taken as predictors; for this model the remaining parameters were omitted in the course of analysis as non-informative, i.e. very weakly correlated. The parameters with weights > 0.3 were assumed to be correlated. The association between two parameters was quantified by calculating their correlation weights in such a way that for specific pairs of the considered parameters, the algebraic product of their corresponding weights and the cosine of the corresponding angle, i.e. the angle determined by two lines connecting the origin with coordinates of both parameters on the PLS plot, were calculated. The calculations of PLS model were carried out with the package SIMCA-P v.9 (Umetrics, Sweden). The correlation weights were calculated with software delivered by MP System Co. (Poland).

3. Results

The intake of fodder, sulforaphane and total composition of the diets are presented in Table 1. The results for hormone levels, changes in antioxidant status, interleukins and body temperature are collated in Table 2. Descriptive statistics for haematological and biochemical parameters are given in Table 3. The correlation weights based on PLS model are shown in Table 4, while other results of PLS or cluster analysis are to be found in Figs. 1–3.

4. Discussion

In the presented evaluation, three models were implemented, specifically the normal diet and two independent models of thyroid damage based either on iodine deficiency diet, or sulfadimethoxine ingestion. An insignificant tendency for increasing TSH level was observed for the iodine deficiency model, whereas for the sulfadimethoxine one a significant difference was detected ($P < 0.001$) in comparison to the control group. For fT3, a significant reduction ($P < 0.01$) in this hormone concentration was also observed for group S. The ID model is well known for inducing negative changes in the thyroid gland, due to continual decreases in intracellular iodine concentrations. However, no significant differences in the hormone level were detected in our experiment. In a 5 weeks experiment by Ikeda et al. [14] a significant increase in TSH level, a significant decrease in T4 level, and no effect in T3 concentration were observed. An earlier paper by Ikeda et al. [12], reported a 10 weeks experiment where no differences in TSH and T3 levels had been detected, and only T4 concentrations had been found to significantly decrease. A compensatory increase in TSH is recognized as the earliest indicator of hypothyroidism [18], which originates from a negative feedback between TSH and thyroid hormones. No changes in TSH levels as observed in longer experiments (see [12]; present work) may be attributed to the organism adaptive response. The sulfadimethoxine model has been recommended as a good model for thyroid damage, where decreased T3 and T4, as well as increased TSH are mediated by inhibition of thyroid peroxidase (TPO) [14]. In fact, a significant decrease in fT3 and an increase in TSH concentration were observed for group S. The two models for thyroid damage. i.e. ID and S, used in this experiment caused no significant decrease in fT4 level, which is in line with the observation by Schöne et al. [19] who found that neither the content of goitrogens, nor iodine dosage affected the T4 level in older pigs (15 weeks), unlike in young animals (4 weeks pigs), where significant changes in the concentration of these hormones were noted. A similar suggestion in respect of dogs was published by Trepanier [20].

Broccoli sprouts did not cause any significant changes in TSH, fT3, or fT4 levels. To the best of our knowledge our study is the first assessment of a possible influence of broccoli, a well known abundant source of glucosinolates, i.e. chemical compounds with a potential antithyroid effect, on thyroid function [21]. For brassica vegetables, most of the investigations had been conducted many years ago [e.g. 18], therefore, there is little up to date evidence that these vegetables have a significant negative effect on the thyroid function.

For the two factors possibly influencing thyroid function, e.g. iodine deficiency and sulfadimethoxine, both combined with consumption of broccoli sprouts, different effects were recorded. For the BDI group none significant influence was recorded, and broccoli sprouts were found to have an apparent protective effect against thyroid damage as TSH and fT3 respectively decreased and increased significantly, while for fT4 levels no effect was observed in the group of rats receiving sulfadimethoxine (BS). This observation is in agreement with results obtained by Ikeda et al. [14] who indicated that soybean intake neither lead to a deterioration in the effects of SDM treatment, nor ameliorated thyroid lesions. A possible explanation to our results could also be based on the fact that broccoli sprouts may comprise a source of iodine in the animals diet, as it had been proven by our evaluation (data not shown). Regretfully, data on the amount of iodine in sprouts is scarce. According to Haldimann et al. [22], the average concentration of iodine in leafy vegetables reaches 23.6 μg/100 g of dry weight (about 2.4 μg/100 g fresh weight).

The thyroid gland displays several protective mechanisms against oxidation, as the hormone synthesis requires hydrogen peroxide for iodine organification. The crucial role in the thyroid is played by GPX and TR [23] which prevent oxidative stress and induction of apoptosis. Additionally, thyroid hormones are also involved in the regulation of the oxidative metabolism and play a significant role in generating ROS

Table 1
Detailed composition of the diet and the fodder and sulforaphane intake.

	C	DI	B	BDI	S	BS
FODDER COMPOSITION [g/kg fodder]						
Gluten	200	200	200	200	200	200
Sprouts	–	–	70	70	–	70
L-cystein	3	3	3	3	3	3
Starch	532	532	462	462	532	462
Sucrose	100	100	100	100	100	100
Corn oil	70	70	70	70	70	70
Cellulose	50	50	50	50	50	50
AIN-93G[a]	35	–	35	–	–	–
AIN-93G[b]	–	35	–	35	35	35
AIN-93G[c]	10	10	10	10	10	10
Total g	1000	1000	1000	1000	1000	1000
INTAKE OF FODDER [g/day]						
Fodder intake	14.8 ± 3.3^{abc}	11.75 ± 3.0^{e}	11.6 ± 3.0^{ad}	14.0 ± 3.2^{f}	23.1 ± 3.8^{befg}	11.1 ± 2.5^{cdg}
INTAKE OF SULFORAPHANE [mg/day/rat][d]						
Sulforaphane intake	–	–	0.9 ± 0.2^{h}	1.1 ± 0.2^{hi}	–	0.9 ± 0.2^{i}

Mean values with the same superscript are significantly different between the indicated group at $P < 0.05$ (h); $P < 0.01$ (a,c,i); $P < 0.001$ (b,d,e,f,g).
[a] Standard mixture of essential and trace elements.
[b] Standard mixture of essential and trace elements without iodine.
[c] Standard mixture of vitamin.
[d] Average amount of sulforaphane after hydrolysis of glucoraphanin.

[24]. In our experiment, no significant changes in GPX3 and GPX1 activity were observed apart from a slight increase in GPX1 activity in groups B, DI, and BDI vs. C, but for TR activity a significant increase was observed in groups B vs. C, BDI vs. C, BDI vs. BS and BDI vs. S. This observation is in agreement with Clarke et al. [25] who demonstrated that sulforaphane may increase TR and GPX activity, and that it is one of the supportive mechanisms in live organisms against negative agents. In iodine deficiency, or in the presence of antithyroid agent (DI or S), broccoli sprouts exert even a beneficial influence on the antioxidant balance of the thyroid gland. Additionally, FRAP was significantly higher in the group with sulfadimethoxine and broccoli sprouts (BS) in comparison to the other investigated groups considered as a whole.

Our results indicated that neither addition of broccoli sprouts to the diet, nor sulfadimethoxine or iodine deficiency exposure, caused significant changes in RBC and MCH parameters. Additionally, with PLS model applied correlations between Hct and FRAP (CW = 0.118), Hb and Hct (0.132), Hb and FRAP (0.134) were found. A similar observation was made by Woyengo et al. [26] who found that an increase in dietary levels of canola (*Brassica napus* L.) did not adversely affect blood haemoglobin and hematocrit in broilers. Sprouts caused a significant decrease in WBC and PLT levels in comparison to the control group. In a human study, Munters et al. [27] also noted that consumption of 20 g broccoli sprouts for 4 days caused a significant reduction in the amount of lymphocytes, and in the percentage of monocytes. In our evaluation WBC was found to be highly positively correlated with TG concentration (CW = 0.340) and negatively correlated with creatinine (CW = −0.104), Hct (CW = −0.124), FRAP (CW = −0.125), and Hb (CW = −0.187). A negative correlation between WBC and FRAP may indicate that changes in the amount of WBC could be the organism response to high concentration of antioxidant compounds in the evaluated material.

For thrombocytes we have not found any data on the broccoli influence to decrease the amount of such cells, whereas sulforaphane displays antiplatelet activity and may initially activate adenylate cyclase, as it ultimately inhibits platelet aggregation and thrombotic formation [28]. In the model of thyroid damage, most of the significant changes were observed in animals with a deficiency of iodine, namely a significant increase in Hb, Hct, MCV, and a significant decrease in MCHC, WBC, PLT, rather than sulfadimethoxine for which only a significant decrease for WBC was recorded. The observation related to

Table 2
Hormone levels (TSH, fT3, fT4), antioxidant status parameters in plasma, thyroid glands, body temperature and IL-6, IL-10 concentration for the investigated animals.

Parameters	C	B	DI	BDI	S	BS	P value
HORMONES							
TSH [μlU/L] n = 12	9.14 ± 1.55^{a}	9.81 ± 1.95^{b}	10.43 ± 2.71^{c}	8.33 ± 1.21^{d}	26.85 ± 11.01^{abcde}	11.71 ± 5.31^{e}	a,b,c,d,e***
fT3 [pg/mL] n = 12	4.33 ± 0.70^{a}	4.65 ± 0.69^{b}	4.51 ± 0.98^{c}	4.40 ± 1.04^{d}	2.90 ± 0.93^{abcde}	4.18 ± 1.05^{e}	e*a,d**b,c***
fT4 [ng/dL] n = 12	10.77 ± 4.12	9.22 ± 3.07	10.65 ± 1.80	8.00 ± 1.30	9.09 ± 2.80	8.65 ± 2.65	–
THYROID GLANDS							
GPX1 [U/g] n = 4	2.27 ± 0.99	3.97 ± 0.76	3.53 ± 1.23	3.65 ± 1.24	1.53 ± 0.39	2.28 ± 1.38	–
TR [mU/mg] n = 4	2.53 ± 0.13^{ab}	7.13 ± 1.28^{a}	3.50 ± 2.31	5.73 ± 1.65^{bcd}	1.86 ± 0.87^{c}	2.45 ± 0.71^{d}	a,b,c,d*
PLASMA							
GPX3 [U/mg] n = 12	0.49 ± 0.17	0.48 ± 0.05	0.56 ± 0.03	0.52 ± 0.03	0.51 ± 0.04	0.47 ± 0.03	–
FRAP[μmol/L]n = 12	434.64 ± 88.96^{a}	453.40 ± 16.31^{b}	391.75 ± 39.00^{cf}	446.70 ± 48.71^{d}	475.75 ± 89.18^{ef}	573.98 ± 72.83^{abcde}	a,b,c,d***e,f**
IL-6 [pg/ml] n = 6	24.43 ± 17.89	19.86 ± 13.98^{a}	54.73 ± 9.28^{a}	40.46 ± 11.45	23.00 ± 13.78	40.36 ± 26.83	a*
IL-10 [pg/ml] n = 6$^{\#}$	4.33 (0.69, 27.15)	6.64 (0.85, 52.19)	10.08 (1.18, 86.25)	3.16 (0.57, 17.55)	2.67 (0.64, 11.19)	1,82 (0.54, 6.08)	
BODY TEMPERATURE							
TEMP [°C] n = 12	35.5 ± 0.5^{ab}	33.9 ± 0.9	34.3 ± 1.2^{c}	31.9 ± 0.6^{acde}	31.7 ± 1.8^{df}	33.7 ± 1.0^{bdef}	e**a,b,c,d***f*

Mean values with the same superscript are significantly different between the indicated group at *P < 0.05 **P < 0.01 ***P < 0.001.
– reverse logarithm.

Table 3
Blood morphology and biochemical parameters for the investigated animals.

Parameters	C	B	DI	BDI	S	BS	P value
n = 12	BLOOD MORPHOPLOGY PARAMETERS						
RBC [10^6/μL]	9.13 ± 0.59	9.17 ± 0.80	9.88 ± 0.44	9.25 ± 0.78	9.13 ± 0.45	9.48 ± 0.82	
Hb [g/dL]	13.82 ± 0.50^{a}	14.19 ± 1.44^{b}	15.41 ± 0.53^{ab}	14.28 ± 1.26	14.35 ± 0.60	14.42 ± 1.27	b*a**
Hct [%]	44.42 ± 2.56^{a}	46.41 ± 4.91	50.84 ± 2.48^{ab}	47.61 ± 4.46	46.30 ± 2.15^{b}	48.63 ± 4.83	a**,b*
MCV [fL]	48.61 ± 1.83^{abcd}	51.00 ± 1.46^{a}	51.89 ± 0.43^{b}	51.43 ± 1.67^{c}	50.65 ± 2.20^{e}	52.91 ± 2.16^{de}	a,e*,b,c,d***
MCH [pg/cell]	15.19 ± 0.79	15.47 ± 0.43	15.57 ± 0.43	15.39 ± 0.47	15.75 ± 0.86	15.79 ± 0.83	
MCHC [g/dL]	31.19 ± 1.17^{abc}	30.34 ± 0.74	30.04 ± 0.45^{ad}	29.89 ± 0.62^{be}	31.02 ± 0.68^{def}	29.68 ± 0.72^{cf}	a,b**,c,e,f***,d*
WBC [10^3/μL]	20.85 ± 8.80^{abcde}	14.05 ± 4.89^{afg}	8.79 ± 1.32^{bf}	8.61 ± 1.27^{cg}	11.06 ± 2.85^{d}	11.42 ± 2.91^{e}	a**,bcde***,fg*
PLT [10^3/μL]	946.33 ± 202.71^{ab}	664.41 ± 240.79^{a}	582.86 ± 287.34^{bc}	676.85 ± 337.11	889.40 ± 125.43^{c}	704.81 ± 81.04	a,c*,b**
	BIOCHEMCAL PARAMETERS						
Glucose [mmoL/L]	11.51 ± 2.40	12.91 ± 1.05^{a}	11.99 ± 1.09^{b}	10.03 ± 1.40^{ab}	11.80 ± 1.95	11.56 ± 1.63	a**,b*
Uric acid [mg/dL]	22.83 ± 11.22	17.00 ± 4.97	25.75 ± 6.60	23.91 ± 9.46	20.83 ± 5.84	20.50 ± 9.76	–
Urea [U/mg]	8.47 ± 1.35^{a}	8.19 ± 3.01^{b}	11.81 ± 2.88	8.12 ± 1.82^{c}	10.16 ± 2.13^{d}	14.69 ± 7.26^{abcd}	a**,b,c***,d*
Creatinine [μmol/L]	21.50 ± 4.98^{a}	18.75 ± 4.59^{b}	17.75 ± 1.95^{ac}	16.36 ± 1.28^{d}	23.45 ± 4.82^{bcde}	18.58 ± 2.68^{e}	a,c**,b,e*,d***
ASPAT [U/L]	103.20 ± 28.72^{ab}	75.72 ± 33.54	87.00 ± 19.76^{c}	51.92 ± 15.54^{ac}	72.59 ± 16.64^{b}	76.72 ± 19.95	a***,b*,c**
ALAT [U/L]	28.87 ± 11.43^{abc}	63.94 ± 27.17^{a}	85.85 ± 27.57^{bde}	52.21 ± 10.31^{d}	46.73 ± 18.85^{ef}	74.89 ± 15.25^{cf}	a,b,c,e,f***,d,f**
TG [mmoL/L]	0.79 ± 0.14	1.02 ± 0.57^{ab}	0.48 ± 0.14^{ac}	0.91 ± 0.32^{c}	0.63 ± 0.13^{b}	0.67 ± 0.16	a***,b*,c**
TC [mmoL/L]	2.50 ± 0.53^{a}	2.36 ± 0.17	1.95 ± 0.24^{a}	2.25 ± 0.17	2.25 ± 0.59	2.33 ± 0.13	a**
HDL [mmoL/L]	0.88 ± 0.06	0.85 ± 0.17	1.05 ± 0.78	1.16 ± 1.03	0.95 ± 0.50	0.67 ± 0.38	–
PAL [U/L]	104.90 ± 48.64^{abcd}	150.00 ± 25.95^{a}	165.33 ± 26.11^{be}	147.90 ± 14.49^{c}	127.27 ± 34.98^{e}	151.73 ± 21.29^{d}	a,c,d**,b***,e*

Mean values with the same superscript are significantly different between the indicated group at *P < 0.05 **P < 0.01 ***P < 0.001.

Table 4
Correlation weights for the pairs of parameters (based on PLS model).

Pairs of correlated parameters		Correlation weights
WBC	TG	0.340
Creatinine	MCHC	0.230
Creatinine	FRAP	0.159
Creatinine	Hb	0.158
Creatinine	Hct	0.156
Hb	FRAP	0.134
TG	fT3	0.134
Hb	Hct	0.132
Hct	FRAP	0.118
TG	FRAP	−0.109
TG	Hct	−0.109
WBC	Creatinine	−0.104
WBC	Hct	−0.124
WBC	FRAP	−0.125
WBC	Hb	−0.187
TG	Hb	−0.196

WBC in group S is in agreement with Trepanier [20] who indicated that such effects on the blood (leucopoenia) may result either from hypersensitivity reactions, or from hydroxylamine or nitroso metabolites of sulfodimetoxine. Broccoli sprouts introduced into the diet of animals with thyroid damage agent influenced MCV and MCHC in the BS vs. S group.

It had also been suggested that the thyroid gland removal in newborn or young adult rats caused a reduction in the number of peripheral blood lymphocytes and depression of the humoral immune reactions [29]. These immunological defects have been fully established in perinatally thyroidectomised rats after weaning, while in animals thyroidectomised at young-adult age they are observable 45–60 days after the operation. These results stress the importance of thyroid hormones for lymphocyte proliferation either during the period of ontogenetic development or in adult life. However, Christ-Crain et al. [30] proposed that the changes in lymphocytes are due to the autoimmune process *per se*, rather than the lack of the thyroid hormone. The true mechanism remains to be established.

A statistically significant PLS model was constructed for biochemical data. Fig. 1 and Table 4 show the positive correlation between creatinine and MCHC, FRAP, Hb, Hct, as well as between TG and fT3. We also observed strong negative correlations between TG and Hct and Hb.

Though broccoli sprouts did not cause a significant increase in glucose level, such a tendency could be observed. However, for iodine deficient rats, their sprouts enriched diet caused a significant decrease in glucose, which happens to be a beneficial effect. On the other hand, El Hendy et al. [31] observed an increase in glucose level in hyperglycaemic rats following consumption of 10% broccoli enriched fodder under an 8 week experiment and a positive effect on the glucose concentration was observed only for the diet containing 30% of broccoli sprouts [31]. For lipid parameters, such as TC, TG, or HDL, the greatest changes were observed for TG. They were found to be correlated with fT3 concentration and observed in iodine deficient rats fed with broccoli. TC decreased significantly only in group DI vs. C. HDL was unaffected. Okulicz et al. [32] evaluated the influence of sulforaphane (10 mg/kg) administered intragastrically once a day over 2 weeks on the lipid profile in rats and found an increase in HDL, and TG in the liver by 29% and 16%, respectively, whereas serum TG and TC remained unchanged. Overall, our evaluation of the influence of broccoli on lipid profiles is not fully compliant with the papers published previously where a reduction in plasma triglyceride, LDL-cholesterol, and total cholesterol [33,34] have been shown. The reason for such divergences may be associated with the different metabolic models applied, or attributed to the different amount of broccoli sprouts used in the experiments. Additionally, our results are in contrast with those of Messarah et al. [35] where the influence of benzythiouracil in animal model lasting 5 weeks was evaluated. The most plausible explanation may be that our experiment lasted 8 weeks, so an adaptive mechanism to metabolic disturbances may have been initiated. Such an effect was also suggested for thyroid damage by Ikeda et al. [12].

Liver parameter ALAT was increased in rats fed with broccoli fodder and this effect could be explained by the induction effect on the CYP activity. In the short-term experiment conducted by Gaona–Gaona et al. [36] where sulforaphane was injected intraperitoneally at a dose of 500 μg/kg/d over 3 days, and where no changes in ASPAT or ALAT were observed. Galati & O'Brien [37] investigated the toxicity of natural compounds such as propyl gallate, gallic acid, and epigallocatechin-3-gallate in vivo in mice, and found that all three compounds caused significant increase in plasma ALT levels characteristic for liver injury, which could suggest free radical formation and pro-oxidant toxicity. This is in agreement with Perocco et al. [38] who indicated that glucoraphanin, a sulforaphane precursor, may cause induction of the cytochrome P450 (CYP) isoforms CYP1A1/2, CYP3A1/2 and CYP2E1, and who also found the compound to have generated a large amounts of

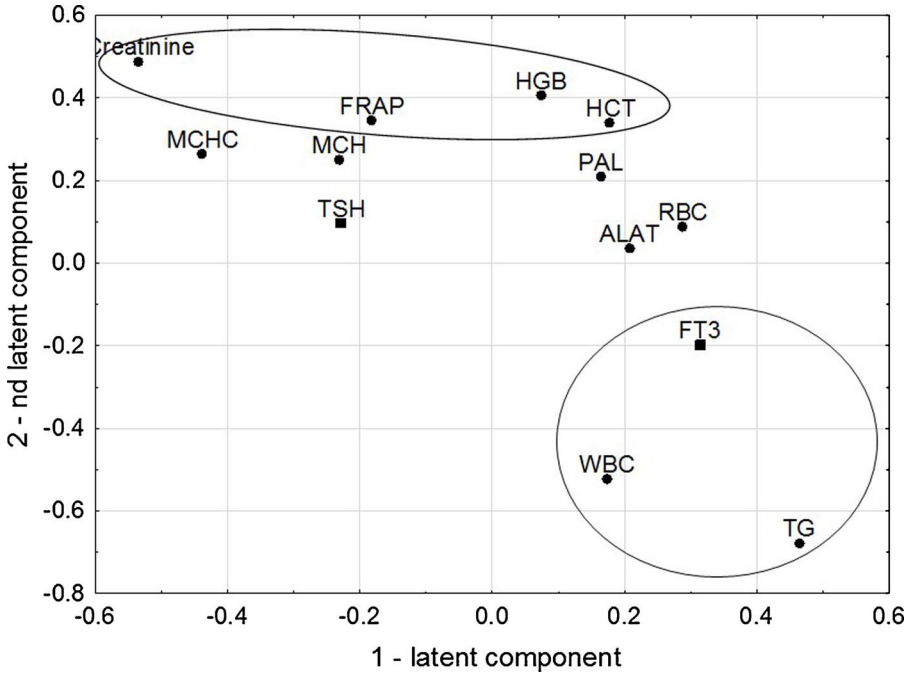

Fig. 1. Parameter loadings on the first two principal components.

various reactive radical species. Perocco et al. [38] suggests that long-term uncontrolled administration of glucoraphanin could pose a potential health hazard because many carcinogens are metabolically activated by CYP1A2. In all our animal groups we observed a decrease in ASPAT when compared to the control animals. In a similar model of hypothyroidism where the thyroid dysfunction was induced by benzythiouracile, no significant changes in liver parameters were observed [35].

There were no differences in uric acid level, which provides valuable information for hyperuricemia patients. An increasing tendency in uric acid concentration was observed for models of thyroid damage (DI, S) and this was particularly significant in group BS vs. S. An increase in plasma creatinine level has been used to measure the chronic renal failure when kidneys are not able to eliminate protein metabolic by-products [39], however, this parameter tend to decrease in all groups in comparison to the control one, with one insignificant exception (S). We assumed the animal kidneys in the experiment to have worked properly.

To our knowledge, there is no relevant reference in the literature regarding the decrease in alkaline phosphatase activity as observed in our experiment in broccoli fed groups with a thyroid damage. Protein malnutrition is often associated with an increased activity of alkaline phosphatase [39], but as this enzyme is non-specific and, moreover, none negative changes in animal functioning were observed throughout the experiment, the interpretation of such results remains ambiguous.

The distribution of examined animals in the space determined by the first two latent components showed the existence of several relatively distinct and homogenous clusters, reflecting animals belonging to

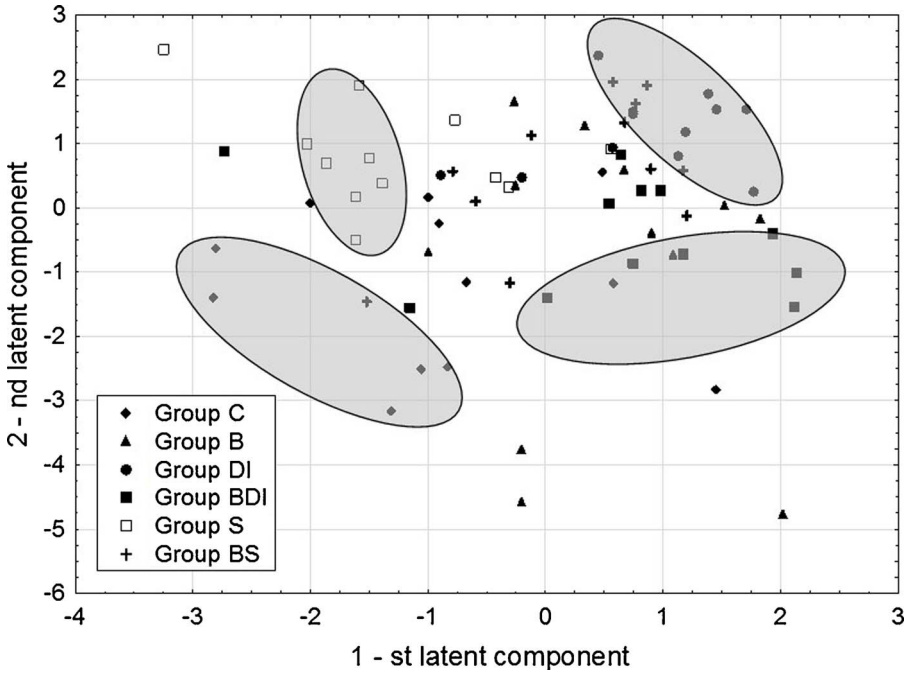

Fig. 2. The distribution of the examined animals in the space determined by the first two principal components.

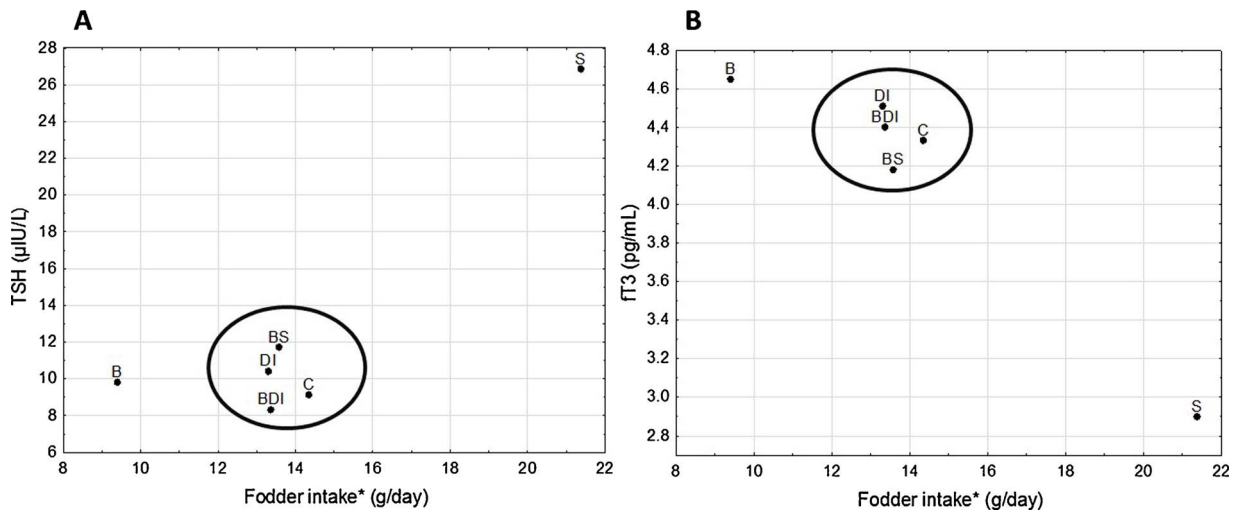

Fig. 3. Scatterplot of studied groups of animals in the space determined by either TSH (A) or fT3 (B) and fodder intake (* – mean values for last three days preceding the end of experiment).

the experimental groups (Fig. 2). True positive rates for the apparent clusters were as follows: C 45%, DI 82%, BDI 50%, S 58%. Only animals from B and BS groups did not form any tight cluster

The next part of our experiment was to evaluate the influence of broccoli sprouts, both alone and combined with thyroid damage factors, on the inflammatory markers. We choose two cytokines, one pro-inflammatory (IL-6) and one anti-inflammatory (IL-10). A significant increase in IL-6 concentration (Table 2) for group DI vs. B group was recorded. The broccoli sprouts introduced to the diet caused an insignificant decrease in IL-6 (B vs. C; BDI vs. DI). Different models of thyroid damage applied in the experiment resulted in different effects on IL-6 level, including an increase in iodine deficiency group and a decrease in the sulfadimethoxine group. No significant differences in IL-10 concentration were found. By means of gamma statistic we did not find a significant correlation between IL-10 and IL-6. The effect of broccoli sprouts on cytokines may be attributed to many different chemical compounds present in this material, not to sulforaphane alone, but also to other phenolic compounds. In vitro studies have confirmed the anti-inflammatory effect of sulforaphane [40,41], and in vivo evaluations of this aspect are sparse. Mirmiran et al. [42] evaluated the effect of broccoli sprouts in different doses in patients with type II diabetes and did not find any significant differences in TNF alpha and IL-6 levels.

The results for changes in thyroid homeostasis reported above are additionally supported by evaluated rats body temperature (see Table 2) and increased fodder intake (Table 1). Neither broccoli sprouts diet, nor iodine deficiency diet, caused significant changes in the rats body temperature. Significant decrease for this parameter was observed in the control rats in comparison to the following groups: BS ($P < 0.001$), and BDI ($P < 0.001$). The lowest body temperature was observed in group S (31.7 ± 1.8 °C) for which also fT3 level was found the lowest when compared to all the investigated animals. Thyroid hormone fT3 is responsible for increasing the metabolic rate by inducing transcription of uncoupling protein 1, a major component of the thermogenic program and a specific marker of thermogenic adipocytes in the brown adipose. T3 works by activating the central nervous system to induce thermogenesis [43]. Broccoli sprouts added to the DI led to a larger temperature drop, which indicates that sprouts intake enhances the effect of iodine deficiency, but the opposite pattern was observed in the group of rats that received sulfadimetoxine, where positive effect of sprouts, namely a significant increase in the body temperature was observed (BS vs S).

Selected groups of food products involving red pepper, black and white pepper, as well as ginger containing different active compounds such as capsaicin, piperine, gingerols (shogaol, and 6-paradol) [44] have been proven to affect thermogenesis. All of these substances are known to act as agonists for TRPV1 receptor, and as such are expected to activate thermogenesis and reduce body fat. Intensified thermogenesis in rodents was also observed by Masamoto et al. [45] via TRPA1 activation by allyl-isothiocyanates and benzyl-isothiocyanates present also in brassica vegetables, particularly in Japanese horseradish, but the mechanism for broccoli should be investigated further.

Despite the fact that the average amount of sulforaphane obtained after hydrolysis of glucoraphanin was significantly higher ($P < 0.01$) in BDI group (1.1 mg/day/rat) than in BS (0.9 mg/day/rat), in the latter group a concurrent positive effect of broccoli sprouts intervention (BS vs B; $P < 0.001$) on the fodder intake was observed to take place (Table 1). Such observations may be supported by effects of isothiocyanates on the increase of thermogenesis as mentioned above, as well as by the confirmed relationship between fodder intake and the level of TSH and fT3 which showed that increased intake of fodder was associated with increased TSH level and decreased fT3 level. Visual inspection of Fig. 3A and B showed that 4 groups (C, DI, BDI, BS) formed one tight cluster and indicted that iodine deficiency groups did not differ from group C, but broccoli sprouts neutralize the negative effect of sulfadimetoxine on the thyroid. Sprouts added to the diet alone had a positive impact on the thyroid parameters.

5. Conclusions

Due to unavailability of reports on similar investigations, evaluation of the influence of widely popular broccoli sprouts on the thyroid function seems to be essential and timely.

Broccoli sprouts have been confirmed not to have any harmful effects on the thyroid homeostasis in animals in terms of TSH, fT3 and fT4 levels which remained unchanged. For animals with hypothyroidism, broccoli sprouts exert a beneficial influence on the antioxidant balance of the thyroid gland. The plant may also display a supportive potential on the thyroid gland damage caused in particular by sulfadimetoxine via TR and GPX activity. It may even increase body temperature.

Broccoli sprouts intake by healthy rats decreased white blood cells and thrombocytes and adversely interfered with liver function in rats, most likely due to increased dietary concentration of sulphur compounds delivered with sprouts.

Our results strongly suggest that neither addition of broccoli sprouts to the diet, nor sulfadimethoxine or iodine deficiency exposure, cause negative changes in red blood cells parameters, glucose level, uric acid or kidney function.

Broccoli sprouts diet caused a significant decrease in IL-6 in comparison to the iodine deficient rats.

It is worthwhile to note that the obtained results related to the thyroid function and others parameters remain valid for male rats, and that their interpretation in relation to human exposure to broccoli products should be carefully considered.

Therefore, further detailed investigation particularly with regard to the amount of sprouts, time of exposure, and different thyroid diseases is needed.

Conflict of interest

The authors declare no competing financial interest.

Ethical approval

The protocols for animal experiments were approved by the Animal Experimentation Committee of Jagiellonian University, Kraków, Poland. The animal experiment was carried out in accordance with the NIH guide for the care and use of laboratory animals.

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