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Interaction between iodine and glucosinolates in rutabaga sprouts and selected biomarkers of thyroid function in male rats☆

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ABSTRACT

Rutabaga sprouts belong to the *Brassicaceae* family and may exert a negative influence on thyroid function, because they are a rich in glucosinolates. These sprouts are also valuable source of iodine (6.5 ± 0.6 μg/100 g of fresh weight). Sprouts were tested in a long-term experiment with young male rats as an element of their diet, combined with two models of hypothyroidism, the first – deficit of iodine and the second – sulfadimethoxine ingestion as a pharmacological agent caused inhibition of thyroid peroxidase. Evaluations were performed for the serum TSH and thyroid hormones together with analyzes of thyroid histopathology, cytosolic glutathione peroxidase (GPX1), thioredoxin reductase in the thyroid, plasma GPX3 and CAT, erythrocyte GPX1. Rutabaga sprouts' intake by healthy rats did not cause any harmful effect on their health, including thyroid function. For animals with hypothyroidism, rutabaga sprouts enhanced the adverse effect of iodine deficiency or ingestion of sulfadimethoxine on the organism. According to the results obtained for young male rats thyroid function, the interpretation of data for human exposure to rutabaga sprouts has to be avoided. Furthermore, unless new scientific data confirms a lack of the negative effect of brassica sprouts on thyroid function in human, they should not be excluded from the group of goitrogenic products.

1. Introduction

Rutabaga (*Brassica napus* L. var. *napobrassica*) sprouts have been recognized as a novel functional food with a proapoptotic profile and a source of compounds of different biological activity [1,2]. Rutabaga roots have been an element of the human diet for years, but unfortunately during the last decades these vegetables have been gradually forgotten. Pennington and Fisher [3] included rutabaga in one of the ten fruit and vegetable subgroups which have unique concentrations of components and should be used by dieticians in the preparation of daily menus. Brassica vegetables possess anticancer activity [4,5] and mostly sulfur compounds (such as glucosinolates) and polyphenols are responsible for this [6]. On the other hand, it has long been known that these compounds can be responsible for impairment of thyroid gland function, particularly in poultry, pigs and rats [7–10]. Hence, strong warnings about the negative effects of brassica products on thyroid function are presented in many guide books used in human nutrition. Unfortunately, such nutritional suggestions have been often based on insufficiently documented data, and after being repeated many times they have become considered as confirmed recommendations with biological plausibility. Therefore, in this respect new methodologically reliable investigations are strongly required to evaluate clinically relevant outcomes. Also the safety aspects of rutabaga sprouts should be evaluated. Thus, the present study of rutabaga sprouts was the first to be undertaken as a part of a general survey of these vegetables.

The main aim of this investigation was to study the effects of rutabaga sprouts on the thyroid gland and on general function of the male rat organism. During the experiment three independent models were used. The first one was the normal diet. The second model was based on a diet with iodine deficiency causing thyroid hyperplasia by an increase of TSH levels and the reduction of thyroid hormone levels [11,12]. The last model was based on sulfadimethoxine (SDM) added to the animal drinking water as an ingredient (0.025%) causing thyroid damage by inhibiting thyroid hormone synthesis due to inhibition of thyroid peroxidase (TPO) [13].

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The first analytical step of the study was to employ the UPLC–MS/MS method to evaluate the composition of glucosinolates (GS) and iodine concentration in rutabaga sprouts and to establish whether these new products may cause health problems. The second objective was to investigate the rats' response to the presence of rutabaga sprouts in their diet, combined with a deficit of iodine or with ingestion of sulfadimethoxine. Animal response criteria were: the serum concentration of thyroid-stimulating hormone (TSH) and free thyroid hormones (triiodothyronine (fT3), thyroxine (fT4)); histopathological analysis of the thyroid tissue; cytosolic glutathione peroxidase (GPX1) and thioredoxin reductase (TR) activity of the thyroid; ferric reducing ability of plasma (FRAP), plasma GPX3 and catalase (CAT) activity; and finally GPX1 activity of red blood cells of animals after an extended experiment (8 weeks).

2. Materials and methods

2.1. Plant material

Rutabaga seeds (*Brassica napus* L. var. *napobrassica*) were collected from plants harvested in eastern Poland (Zamość) in 2012. Specimen copies were deposited with the Małopolska Plant Breeding Company sp. z o.o. and the Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Jagiellonian University Medical College (No#BNN/PP/PL 1024). Eight day old sprouts were harvested by the Uniflora Company, Poland. The time of rutabaga sprouting was chosen by scrutinizing the parameters of antioxidant and proapoptotic activities of sprouts obtained and published previously [1]. After sprouting, these materials were lyophilized (Liogam Company, Poland) to obtain dry material suitable for preparation of animal fodder.

2.2. UPLC–MS/MS analysis of glucosinolates

2.2.1. Preparation of samples

After the lyophilization, rutabaga sprouts were frozen and stored at −30 °C. Sprouts were extracted using hot 70% methanol for 3 h. 10 µL of solution was taken from each sample, 10µL of 100 µg/mL chloramphenicol standard was added and the solution was diluted to 1 mL with water to produce dilutions of the stock solutions of the samples. Each dilution was analyzed in triplicate with UPLC–MS/MS.

2.2.2. Quantitative UPLC–MS/MS analysis

The UPLC–MS/MS system consisted of a Waters ACQUITY® UPLC® (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were carried out using the Acquity UPLC BEH (bridged ethyl hybrid) C18 column, 2.1 × 100 mm, and 1.7 µm particle size. The column was maintained at 40 °C, and eluted under the following conditions: 100% of eluent A over 2 min, linear gradient elution from 100% to 50% of eluent A over 3 min, linear gradient from 50% to 0% of eluent B over 2 min, at a flow rate of 0.3 mL/min. Eluent A: water/formic acid (0.1%, v/v); eluent B: acetonitrile/formic acid (0.1%, v/v). Total volume of 10 µL of each sample was injected in triplicate.

The Waters TQD mass spectrometer was calibrated for quantitative analysis using chloramphenicol solution at a concentration of 10 µg/mL, flow 20 µL/min and mixture of eluent A and B 1:1 (v/v) at flow 0.28 mL/min. Optimized settings were as follows: source temperature 150 °C, desolvation temperature 350 °C, desolvation gas flow rate 600 L/h, capillary potential −4.00 kV, collision gas flow 0.1 mL/min. Cone potential and collision energy were individually optimized for each transition. Nitrogen was used as the nebulizing and drying gas. Argon was used as the collision gas. Traces of glucosinolates were analyzed using the Multiple Reaction Monitoring (MRM) method. All analytical data were processed using MassLynx V4.1 software (Waters Corporation, Milford, MA, USA). The average value was expressed as mg or µg/100 g of fresh weight (fw) (Table 1).

Table 1
Concentration of investigated glucosinolates and iodine in rutabaga sprouts (8 days; n = 3) used during experiments with rats.

Compounds	Concentration
Glucosinolates	
Progoitrin [mg/100 g fw]	212.4 ± 12.2
Sinigrin [µg/100 g fw]	154.2 ± 12.3
Glucoerucin [µg/100 g fw]	334.2 ± 81.2
Iodine [µg/100 g fw]	6.5 ± 0.6

2.3. Iodine determination

The concentration of iodine in the extracts of rutabaga sprouts was determined using a modified Sandell-Kolthoff method [14]. This method is based on a color reaction between Ce(IV) and As(III). The absorbance was measured using a UV–VIS Helios-a spectrometer (Spectronic Unicam, Leeds, UK) at 420 nm wavelength. Analyzes of rutabaga sprouts water extracts were repeated three times. Then, the average value was estimated and expressed as µg/L and recalculated into µg/100 g of fresh weight (Table 1).

2.4. Animals

The 72 male (mean weight 123 ± 9 g) 4-week-old Fischer (F344/DuCrI) rats (Charles River Germany) 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 (temperature 22 ± 2 °C, with a relative humidity of 50 ± 5%, 12 h periods of light and darkness). After 1 week of acclimatization, rats were divided into 6 groups, each consisting of 12 animals and fed one of the following diets: standard diet (C); iodine deficiency diet (DI); diet with 7% of lyophilized rutabaga sprouts (R); iodine deficiency diet with 7% of lyophilized rutabaga sprouts (RDI); standard diet with 0.025% SDM administered in animal drinking water (S); or a diet with 7% of lyophilized rutabaga sprouts and with 0.025% SDM administered in their drinking water (RS). The rats had unlimited access to fodder and water. Diets were prepared by The Morawski Fodder Company (Poland), casein used in the diets was replaced with gluten to avoid possible contamination with iodine, all remaining compounds present in the diets were certified as being without iodine. Detailed descriptions of the composition of the diets and intake of the fodder and evaluated glucosinolates are presented in Table 2. 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. After 8 weeks, blood was collected from the abdominal aorta under thiopental anesthesia for hormone assays and other parameters' determinations. Before analyses it was stored at −80 °C. Thyroid glands were divided to be analyzed for the following parameters: GPX1 and TR activities and for histopathological examination.

2.5. TSH, fT3, fT4 analysis

Thyroid hormone analyzes of serum free T4 (fT4), free T3 (fT3) levels 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 conducted for all rats in all groups. The concentrations of fT4, fT3 and TSH were presented as ng/dL, pg/mL and µlU/L, respectively.