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Multidirectional Time-Dependent Effect of Sinigrin and Allyl Isothiocyanate on Metabolic Parameters in Rats

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Abstract Sinigrin (SIN) and allyl isothiocyanate (AITC) are compounds found in high concentrations in Brassica family vegetables, especially in Brussels sprouts. Recently, they have been used as a nutrition supplement for their preventive and medicinal effect on some types of cancer and other diseases. In this research, nutritional significance of parent glucosinolate sinigrin 50 μmol/kg b. w./day and its degradation product allyl isothiocyanate 25 μmol/kg b. w./day and 50 μmol/kg b. w./day was studied by the evaluation of their influence on some parameters of carbohydrate and lipid metabolism in an animal rat model *in vivo* after their single (4 h) and 2 weeks oral administration. Additionally, the aim of this trial was to evaluate the direct action of AITC on basal and epinephrine-induced lipolysis in isolated rat adipocytes at concentration 1 μM, 10 μM and 100 μM *in vitro*. Sole AITC after 4 h of its ingestion caused liver triacylglycerols increment at both doses and glycaemia only at the higher dose. Multiple SIN treatment showed its putative bioconversion into AITC. It was found that SIN and AITC multiple administration in the same way strongly disturbed lipid and carbohydrate homeostasis, increasing esterified and total cholesterol, free fatty acids and lowering tracylglycerols in the blood serum. Additionally, AITC at both doses elevated insulinaemia and liver glycogen enhancement. The *in vitro* experiment revealed that AITC potentiated basal lipolysis process at 10 μM, and had stimulatory effect on epinephrine action at 1 μM and 10 μM. The results of this study demonstrated that the effect of SIN and AITC is multidirectional, indicating its impact on many organs like liver as well as pancreas, intestine *in vivo* action and rat adipocytes *in vitro*. Whilst consumption of cruciferous vegetables at levels currently considered "normal" seems to be beneficial to human health, this data suggest that any large increase in intake could conceivably lead to undesirable effect. This effect is potentiated with time of action of the examined compounds, whose influence is rather adverse for the majority of metabolic pathways (liver steatosis at short duration and insulinaemia, cholesterolaemia at long time treatment). Beneficial action of AITC concerned intensified hydrolysis of TG in the blood serum with a simultaneous lipolysis in adipocytes.

Abbreviations

ACAT	acyl-CoA:cholesterol acyltransferase
AITC	allyl isothiocyanate
b. w.	body weight
BSA	bovine serum albumin
CHL	cholesterol
FFA	free fatty acids
GLS	glucosinolates
HDL	high density lipoproteins
IC_{50}	half maximal inhibitory concentration
iNOS	inducible nitric oxide synthase
ITCs	isothiocyanates
LPL	lipoprotein lipase
NAC	N-acetylcysteine
NO	nitric oxide
SIN	sinigrin
TG	triacylglycerols

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Introduction

More and more investigations have been performed recently on plants in order to discover their possible anticancer properties. It

has been estimated that diets rich in phytochemicals can significantly reduce cancer risk by as much as 20% [15]. Based on epidemiological observations, which correlated reduced incidence of cancer (particularly colon and rectal) in populations with intake of Brassicaceae family vegetables, biological studies have dealt with glucosinolates like sinigrin (SIN) and its hydrolysis product—allyl isothiocyanate (AITC). The conversion of SIN into AITC is catalyzed by myrosinase, a thioglucosidase (E.C. 3.2.3.1) after plant cellular damage or in the presence of gut microflora [10, 21].

Human exposure to these compounds is undoubtedly widespread and frequent, as many common cruciferous vegetables are a rich source of them (*e.g.* Brussels sprouts, red, Savoy, white cabbages, cauliflower, broccoli, condiments and salad crops) [38]. The content of SIN per 100 g fresh weight of appropriate vegetables is in Brussels sprouts (44.50 mg), Savoy cabbage (17.06 mg), white cabbage (16.31 mg), red cabbage (3.77 mg), kale (12.47 mg), cauliflower (4.74 mg), and broccoli (0.19 mg) [38]. According to Steinbrecher and Linseisen [38] the mean total glucosinolate intake, for instance, in a German population (±standard error) was 14.2 (±1.1) mg/day for men and 14.8 (±1.3) mg/day for women. However, allyl isothiocyanate may be present in following foods per 100 g: syrups (1–8.8 mg), meats (8.7 mg), condiments (5.2 mg), baked goods (0.52 mg), candies and ice cream (0.05 mg) [24], mainly as a component in volatile oil of mustard, which is used in pickling spices (meat) and imitation pineapple flavouring (ice cream). Average human consumption of AITC has been estimated to be less than 1 mg/day (approximately 10 μg/kg body weight) [20, 41].

Biological activity of SIN and AITC is wide. Although SIN itself is not known to possess antimicrobial properties, its hydrolysis product AITC kills bacteria and fungi. Herein, AITC action appears to resemble polymyxin B, which is known to bind to cell membrane and to increase its permeability [25]. Also, insecticidal properties of these compounds have been reported [29]. AITC was also shown to significantly inhibit both thioredoxin reductase and acetate kinase at approximately 100 μM [27], enzymes playing an important role in cell growth and proliferation. Although sinigrin itself is not known to show any antiproliferative activity, AITC inhibits proliferation of various types of human cancer cells, with the IC$_{50}$ values at the low micromolar range. Inhibition of cell proliferation by AITC was associated with cell cycle arrest and/or induction of apoptosis [14]. What more, Hwang and Lee [18] reported that AITC and its NAC conjugate play important roles in cancer metastasis by inhibition of cell adhesion, migration and invasion. AITC was also found to significantly inhibit the production of nitric oxide (NO) and the expression of inducible nitric oxide synthase (iNOS)—important signalling molecules in inflammation and cancer [19]. Additionally, AITC induces enzymes of xenobiotic

metabolism in cell cultures and, when included in the diet, in the livers and peripheral organs of mice and rats [6]. AITC has been shown to induce several phase 2 enzymes including NAD[P]H:quinine oxidoreductase-1, glutathione S-transferase in both cultured cells *in vitro* and animal tissues *in vivo* [17, 30]. AITC also was found to significantly inhibit in a dose-dependent manner the formation of gastric lesions induced by ethanol, hydrochloric acid, ammonia, aspirin and indomethacin in Sprague-Dawley rats at the oral dose levels of 1.25–10 mg/kg b. w. (12.5–100 μmol/kg b. w.) [28]. Nevertheless, some data suggest cytotoxic and genotoxic properties of AITC. Allyl isothiocyanate significantly induced 8-oxodG formation in HL-60 cells, but not in H_2O_2-resistant HP100 cells, suggesting the involvement of H_2O_2 in cellular DNA damage [31]. Participation of reactive oxygen species in the genotoxic effect of AITC was also confirmed by the findings reported by Kassie and Knasmüller [20].

Summing up, many substances that occur naturally in food plant may modify, in significant way, the metabolic processes in their consumers. However, we still know very little about the metabolic effects of glucosinolates, particularly in relation to liver (function), where AITC was found in the greatest amounts [4]. There have been only a few studies conducted into the metabolism and biochemical activity of individual glucosinolates and their derivatives. It is known that AITC given in oral doses (50; 100; 150 mg/kg b. w.) for only 3 consecutive days increased liver weight and serum aspartate aminotransferase activity [23]. So that, this result demonstrate that a high intake of this phytochemical instead of giving beneficial effects may produce negative results also on the metabolic pathways. As mentioned earlier, dietary consumption levels of SIN and AITC appear to be several orders of magnitude lower (approximately 150 μg SIN/kg b. w. [38] and 10 μg AITC/kg b. w. [41]) than the doses used in animal studies including the present one (20 mg SIN/kg b. w. and 2.5–5.0 mg AITC/kg b. w.). Since epidemiological data indicate that a diet rich in cruciferous vegetables can protect laboratory animals from tumorigenic effects of model carcinogens, overall intake of glucosinolates and their breakdown products in human diet is suggested to be increased (for example by genetic manipulation of plants or by artificial supplementation of the diet). In order to properly define the balance of benefit and risk of their high consumption, the SIN and AITC dose levels used in the preclinical studies, also in the present trial, are far greater than what humans are normally exposed to.

Taking into consideration the above-mentioned information, this research project aimed at expanding our knowledge about the influence of high SIN and AITC intake on carbohydrate and lipid parameters in a rat model at different time of their action and doses of SIN hydrolysis product *in vivo* and *in vitro* conditions. Time-related response of the analyzed compounds was included in this study in order to

evaluate possible time-interval required for sinigrin decomposition as well as AITC metabolic effect intensification.

Materials and Methods

Rats *in vivo* trial were administrated SIN and AITC at appropriate animal doses, which had no impact on food consumption, rat weight, or general appearance and behaviour were used in such doses by many other researchers [4, 8, 10, 36, 37]. It was assumed that 1 mol of the isothiocyanate corresponds to 1 mol of its parent glucosinolate [38].

All chemicals including sinigrin and allyl isothiocyanate were purchased from Sigma Chemical (St. Louis, MO, USA).

The protocols of experiments were reviewed and approved by the Local Ethical Commission for Investigation on Animals.

In Vivo Experiment

Animal Maintenance

The research was conducted on 64 growing male Wistar rats with an initial body weight of 120±5 g. Male Wistar rats were obtained from Brwinów (Poland). The animals were maintained under standard conditions of light (12 h) and temperature (22±2°C) with free access to laboratory pellets and tap water. The rats were fed a complete laboratory diet ("Labofeed", Poland) containing in %: crude protein 17, crude fat 3.5, crude fibre 7 and 12.15 MJ/kg net energy. The trial involved two separate experiments. Before the research, animals were divided into 8 groups (n=8) for the two investigations (4 groups for each one). The first trial involved single oral administration of SIN or AITC at two different doses (groups II, III, IV, respectively). The second trial concerned SIN or AITC gavage for 14 days (groups VI, VII, VIII, respectively). Animals from groups I and V received water and served as controls. Rats from experimental groups II/VI, III/VII and IV/VIII were given intragastrically 50 μmol/kg b. w. sinigrin or 25 μmol/kg b. w. or 50 μmol/kg b. w. allyl isothiocyanate, respectively. SIN and AITC were prepared by solution in distilled water and were given at a dose of 1 mL/100 g b. w. In the first experiment, the compounds were given once and after 4 h the animals were decapitated. In the second research, rats received the substances once a day as a single morning dose for 14 days and the animals were decapitated 4 h after the last intragastric treatment and their blood and liver were sampled.

Measurement of Carbohydrate and Lipid Parameters

The serum was used for the determination of blood insulin, glucose, free fatty acids (FFA), phospholipids, triacylglycer-ols (TG) and total, free, esterified cholesterol (CHL) and total cholesterol in high-density lipoproteins (HDL). In the liver, cholesterol, triacylglycerols and glycogen were determined.

Insulin was assayed radioimmunologically by using the kit specific for rat hormone (Linco Research, St. Charles, MO, USA).

Glucose was colorimetrically assayed by the enzymatic method with glucose oxidase, peroxidase and o-dianisidine [16]. Free fatty acids were determined according to Duncombe [9] and triacylglyceroles were assayed by the method of Foster and Dunn [12]. Total, free and esterified cholesterol levels as well as cholesterol in HDL were measured by the enzymatic method of Richmond [34]. High-density lipoproteins were obtained from blood serum after precipitation of lipoproteins other than HDL using polyethylene glycol 6000 according to Demacker et al. [7]. Phospholipids were determined enzymatically with a kit provided by BioMerieux (France). Liver cholesterol was assayed after extraction of lipids [11] and evaporation of the extract [34]. The amount of liver glycogen was determined after extraction in 30% KOH and hydrolysis with amyloglucosidase. Liver triacylglycerols were assayed after extraction [11].

In Vitro Experiment

Preparation of Adipocytes

After 7 days of adaptation, the male rats weighing 160±5 g were sacrificed by decapitation and the epididymal adipose tissue was taken. Adipocytes were isolated according to the method of Rodbell [35] with minor modifications [39]. The fat tissue removed from 10 rats was pooled, rinsed with saline, cut with scissors and placed in a plastic flask containing Krebs-Ringer buffer (118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.8 mM $NaHCO_3$) with 3 mM glucose, 3% bovine serum albumin (BSA, fraction V), 10 mM HEPES and 2 mg/mL collagenase (EC 3.4.24.3. from *Clostridium histolyticum*, type II). Incubation was performed for 90 min by shaking at 37 °C. Isolated adipocytes after incubation were rinsed 4 times with warm (37 °C) Krebs-Ringer buffer without collagenase, filtered through nylon mesh and counted under the microscope with a Bürker-Türk counting chamber. Cells viability was about 95% as determined by trypan blue exclusion.

Lipolysis

Fat cell suspensions (10^6 cells/mL) were incubated in plastic tubes at 37 °C for 90 min with the Krebs-Ringer buffer containing 3 mM glucose, 10 mM HEPES and 3% BSA in the absence or the presence of 1 μM epinephrine. The examined AITC was dissolved in DMSO and 10 μL of

this solution (or DMSO alone in the case of control tubes) was added to 990 µL of the buffer with adipocytes. The final concentrations of AITC were 1 µM, 10 µM and 100 µM. Each experiment was performed three times with five replicates. The glycerol released from adipocytes, reflecting the intensity of lipolysis, was measured using the method of Foster and Dunn [12].

Statistical Analysis

The results were evaluated statistically using one-way analysis of variance (ANOVA) and Duncan's multiple range test at $p \leq 0.05$.

Results

Effects of SIN and AITC on Carbohydrate and Lipid Metabolism *In Vivo*

In the short term trial (4 h), the only metabolic changes were observed after AITC administration. The single treatment of allyl isothiocyanate caused increment of glycaemia (34.1%) at higher dose and liver triacylglycerols at both doses (40.6% and 40.2%, respectively). On the other hand, both compounds failed to affect the blood serum insulin, free fatty acids, phospholipids, triacylglycerols, total, free and sterified cholesterol, HDL-cholesterol concentrations, and cholesterol and glycogen contents in the liver (Table 1).

Surprisingly, the 2 weeks experiment brought many significant metabolic changes caused by AITC as well as SIN. In addition, the same mode of metabolic pathway action was observed after administration of both compounds. These changes concerned the alteration of blood serum parameters: FFA, esterified CHL, total CHL and TG. As a consequence of their ingestion, the concentration of FFA, esterified CHL and total CHL was significantly elevated, whereas TG were reduced. A very strong FFA increase was worth nothing (SIN$_{50}$ 138.5%; AITC$_{25}$ 161.5%; AITC$_{50}$ 130.7%). Additionally, AITC at both doses caused considerable increase in the serum insulin concentration (AITC$_{25}$ 63.2%; AITC$_{50}$ 25.0%) as well as glycogen content in the liver (AITC$_{25}$ 28.4%; AITC$_{50}$ 54.1%). Sinigrin showed lack of essential influence on alteration of pancreatic hormone concentration and enhanced glycogen generation, though general increasing tendency to glycogen formation was maintained also after SIN ingestion (Table 1).

Effect of Allyl Isothiocyanate (1 µM, 10 µM, 100 µM) on Basal Lipolysis *In Vitro* Experiment

AITC showed a dose-dependent enhanced effect on basal lipolysis. AITC strongly elevated basal lipolysis by 74.2%

only at 10 µM dose in comparison with the control. Release of glycerol to the incubation buffer reflecting lipolysis remained unchanged at 1 µM and 100 µM AITC dose (Fig 1).

Effect of Allyl Isothiocyanate (1 µM, 10 µM, 100 µM) on Lipolysis Stimulated by Epinephrine (1 µM) *In Vitro* Experiment

The epinephrine-induced lipolysis was dependent on AITC concentration and its stimulatory or inhibitory influence was observed. Lipolysis induced by 1 µM epinephrine was significantly enhanced by AITC at 1 µM (16.3%), although AITC at this dose was not able to stimulate basal lipolysis. AITC at the dose 10 µM revealed universal ability inducing the mentioned basal triacylglycerols decomposition as well as lipolysis in the presence of epinephrine (16.9%). However, in the presence of 100 µM AITC epinephrine-induced lipolysis was clearly decreased, where the 18.6% drop of glycerol release was noted in comparison with the control (Fig. 2).

Discussion

The result of our trials, following different duration of SIN and AITC ingestion shows that SIN degradation product allyl isothiocyanate absorbed from gastrointenstinal track quickly and revealed high biological activity on biochemical parameters. This result is in good agreement with other reports showing that the bioavailability of AITC is extremely high, as nearly 90% of orally administered AITC is absorbed [41]. Pharmacokinetic studies using orally administered isothiocyanates proved that sole glucosinolates degradation products like ITCs undergo rapid absorption from the upper gastrointestinal tract. The rate of appearance of ^{14}C in the blood after dosing rats with [^{14}C]allyl isothiocyanate (25 to 250 µmol/kg) is rapid, with a peak concentration of 10 to 100 µM occurring at 3 h [4]. If it pertains to SIN according to Rabot et al. [33] only about 7% sinigrin can be absorbed from intestinal mucosa; however, it possesses limited biological activity. In treated rats with 50 µmol SIN, the highest recovery of intact sinigrin in the gastrointestinal tract averaged 28 µmol 3 h after gavage. The largest amount, accounting for 46% of the ingested dose, was present in the caecum and colon [10]. These data could explain why, in the present 4-hour trial, metabolic changes were observed only after AITC administration. Higher dose of AITC caused glycaemia, whereas both AITC doses led to liver triacylglycerols gain. Such mode of action in the liver after isothiocyanate ingestion was achieved in the author's former research after 4 h administration of other glucosinolate degradation product like phenethyl isothiocyanate derived from gluconasturtiin [32]. The effect of accumulation of TG in

Table 1 The effect of oral administration of sinigrin and allyl isothiocyanate on some metabolic parameters in the blood serum and the liver after different time of their action

Parameters in the blood serum and the liver	Control	SIN$_{50}$	AITC$_{25}$	AITC$_{50}$
After 4 hours				
Glucose (mmol/L)	3.40±0.31 [a]	3.70±0.21 [ab]	3.87±0.16 [ab]	4.56±0.16 [b]
Insulin (ng/mL)	1.78±0.21	1.52±0.03	1.66±0.10	1.60±0.08
Free fatty acids (mmol/L)	0.45±0.11	0.42±0.03	0.32±0.03	0.37±0.04
Phospholipids (mmol/L)	1.90±0.19	1.95±0.12	2.15±0.17	1.79±0.16
Triacylglycerols (mmol/L)	1.18±0.26	1.66±0.49	1.46±0.26	0.89±0.10
Total cholesterol (mmol/L)	0.84±0.03	0.91±0.03	0.97±0.08	0.90±0.09
Free cholesterol (mmol/L)	0.35±0.03	0.42±0.04	0.40±0.04	0.38±0.06
Esterified cholesterol (mmol/L)	0.48±0.02	0.50±0.04	0.57±0.05	0.53±0.03
HDL-cholesterol (mmol/L)	0.69±0.06	0.70±0.04	0.81±0.09	0.74±0.15
Liver cholesterol (mg/g WT)	1.23±0.05	1.29±0.01	1.16±0.02	1.25±0.04
Liver Triacylglycerols (mg/g WT)	11.53±2.26 [a]	15.49±0.59 [ab]	16.21±1.51 [b]	16.16±0.68 [b]
Liver glycogen (mg/g WT)	31.50±6.77	41.20±4.10	37.23±2.66	33.02±2.44
After 2 weeks				
Glucose (mmol/L)	5.27±0.22	5.07±0.17	5.23±0.17	5.45±0.13
Insulin (ng/mL)	1.52±0.27 [a]	1.41±0.18 [ab]	2.48±0.60 [b]	1.90±0.32 [b]
Free fatty acids (mmol/L)	0.26± 0.01 [a]	0.62±0.03 [b]	0.68±0.05 [b]	0.60±0.05 [b]
Phospholipids (mmol/L)	0.89±0.04	0.96±0.04	1.00±0.04	0.97±0.07
Triacylglycerols (mmol/L)	0.88±0.05 [a]	0.54±0.02 [b]	0.60±0.03 [b]	0.76±0.05 [b]
Total cholesterol (mmol/L)	0.87±0.02 [a]	1.12±0.03 [b]	1.09±0.05 [b]	1.05±0.07 [b]
Free cholesterol (mmol/L)	0.30±0.02	0.28±0.02	0.34±0.04	0.29±0.04
Esterified cholesterol (mmol/L)	0.57±0.01 [a]	0.85±0.02 [b]	0.76±0.02 [b]	0.78±0.04 [b]
HDL-cholesterol (mmol/L)	0.60±0.02	0.74±0.04	0.68±0.05	0.63±0.05
Liver cholesterol (mg/g WT)	2.11±0.04	2.19±0.08	2.13±0.09	2.29±0.07
Liver Triacylglycerols (mg/g WT)	15.73±0.70	17.02±1.05	18.93±2.45	15.31±1.39
Liver glycogen (mg/g WT)	45.00±0.60 [a]	52.30±2.63 [abc]	57.80±3.19 [b]	69.35±4.33 [c]

Values are means±SEM for eight animals. Means in rows with different superscript letters are statistically different ($p \le 0.05$). Sinigrin at the dose 50 μmol/kg b. w. (SIN$_{50}$), allyl isothiocyanate 25 μmol/kg b. w. (AITC$_{25}$) or 50 μmol/kg b. w. (AITC$_{50}$) were given intragastrically

b. w. body weight, WT wet tissue
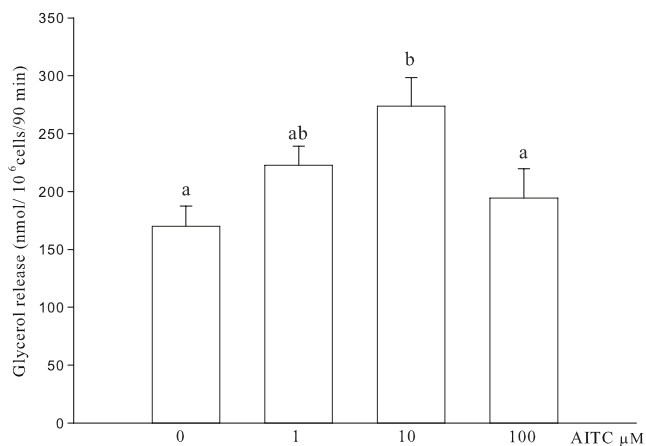
Fig. 1 The effect of allyl isothiocyanate on basal lipolysis in isolated rat adipocytes. Each column represents the mean±SEM for five repetitions (n=5). Mean values marked by different superscript letters differ statistically ($p \le 0.05$).
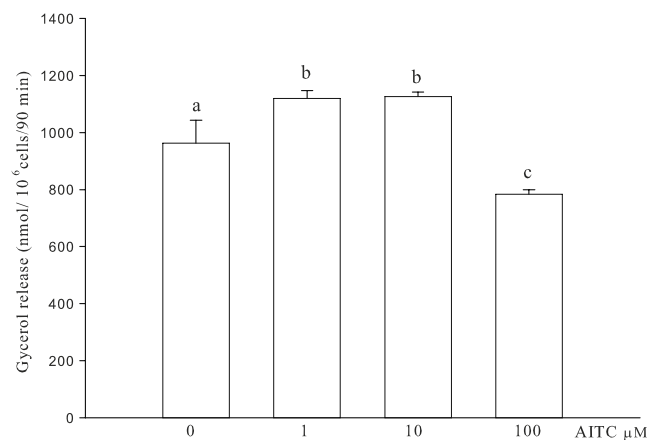
Fig. 2 The effect of allyl isothiocyanate on lipolysis stimulated by epinephrine (1μM) in isolated rat adipocytes. Each column represents the mean±SEM for five repetitions (n=5). Mean values marked by different superscript letters differ statistically ($p \le 0.05$).

the liver may be a result of its enhanced synthesis or/and inhibited utilization by enhanced transfer to a liver cytosolic storage pool. Higher AITC dose caused additionally a distinct blood glucose concentration increase. Because liver glycogen drop with simultaneous insulin elevation was not noted, the disturbed facilitation of glucose entry into muscle, adipose and other tissues could not be excluded after AITC short term trial.

The obtained results from long term action of sinigrin administration may suggest, indirectly, bioconversion of that parent glucosinolate mainly to allyl isothiocyanate because the same mode of metabolic pathway action was observed after SIN as well as AITC administration (the same alteration of FFA, TG, esterified CHL, total CHL in the serum). It has been recently ascertained that the ability to degrade glucosinolates is widely distributed among intestinal flora, which may be the source of enzymes exhibiting the ability to hydrolyze GLS into isothiocyanates [13]. Elfoul et al. [10] showed that AITC appeared in the gastrointestinal tract as soon as 3 h after 50 μmol SIN gavage, but its substantial amounts, up to 100 nmol, were observed really between 9 h and 12 h in the caecal and colonic contents of gnotobiotic rats harbouring a human digestive strain of *Bacteroides thetaiotaomicron*. Such results were achieved also by Krul et al. [21] investigating the microbial metabolism of glucosinolates in dynamic *in vitro* large-intestinal model, closely resembling the human colon. The same alteration in carbohydrate and lipid parameters after long term action of sinigrin as well as allyl isothiocyanate once again confirmed the general observation (agreement) that ITCs are active derivatives of GLS, and the biological activity of the latter depends on the time that related endogenous processes degrade GLS to corresponding ITCs.

In vivo, isothiocyanates are conjugated with glutathione and then sequentially metabolized to mercapturic acids (dithiocarbamates), which undergo the renal excretion [36]. The obtained metabolic changes in liver and serum in the present trial showed that besides the mercapturic acid pathway, isothiocyanates may follow enterohepatic circulation. In rats dosed with (^{14}C) allyl isothiocyanate, the higher value for biliary excretion confirms that some material undergoes such routes of metabolism. In tissues, the greatest amounts of radioactivity are retained in the liver, kidneys and intestinal mucosa [4]. This enterohepatic circulation of allyl isothiocyanate may exert in this way an impact on some intestinal and/or liver enzymes. It is highly probable that the noted elevation of esterified cholesterol was caused by intensified cholesterol esterification by putative AITC influence on acyl-CoA:cholesterol acyltransferase (ACAT; EC 2.3.1.26) activity. ACAT activation in the intestinal cells and/or the liver enhances the uptake of intestinal cholesterol and/or the incorporation of hepatic cholesterol into lipoproteins resulting in elevation of total serum cholesterol [5].

Enhancement of total serum cholesterol observed also in the performed trial could additionally prove this presumption. Because the content of liver cholesterol was unchanged, it seems that AITC influenced only the activity of ACAT in intestinal cells and exerted no impact on liver ACAT. A TG drop was also observed with concomitant exceptionally distinct FFA enhancement in the serum (more than 100%). Because of the above-mentioned enterohepatic circulation of allyl isothiocyanate, it is quite possible that its presence limited chylomicrones synthesis in the intestine and led to the TG drop. However, it may be also amplified by enhancement of lipoprotein lipase activity (LPL; EC 3.1.1.34), which is a critical step in clearance of plasma TG, highly regulated by nutritional and hormonal status. In mammals, the main modulator of LPL is insulin [1]. Enhancement of this hormone in the present research could additionally confirm this assumption. Nevertheless, it is commonly known that the serum FFA are derived mainly from adipose tissue. The elevated supply of fatty acids to circulation by putative action of AITC on adipocytes is proved by the present experiment *in vitro* where AITC potentiated basal lipolysis process at 10 μM. Such AITC concentration in the blood is achieved after dosing rats with 25–50 μmol/kg allyl isothiocyanate [4], the doses also used in the present research *in vivo*. Results of this study revealed also enhanced lipolytic response to epinephrine in adipocytes exposed to 1 μM and 10 μM AITC. Because AITC at 1 μM failed to rise basal glycerol release, this result indicates rather synergistic than additive action with the lipolytic hormone at this dose, amplifying epinephrine effect. The potentiatory effect of AITC on epinephrine-induced lipolysis seems to result, at least partially, from increased cAMP in fat cells, which is crucial for the stimulation of lipolysis [26]. In reverse, action at 10 μM AITC seems to be additive, since this dose was also able to stimulate basal lipolysis. What is interesting, 100 μM AITC strongly attenuated epinephrine signaling pathway and had no effect on basal lipolysis. The stimulation of basal lipolysis and AITC influence on epinephrine activity probably depends on many factors, certainly, on its dose.

Because both AITC doses stimulated significant insulin secretion without any rise in blood glucose concentration, a direct AITC influence not only on liver and intestine, but also on pancreas can be suggested. This organ previously was shown to be affected by glucosinolate breakdown derivatives [40]. It is not also possible to exclude indirect AITC action on this organ by the above-mentioned excessive release of FFA from adipose tissue. According to Boden [3], acute increases in plasma levels of long-chain fatty acids can raise plasma insulin levels by stimulating insulin secretion or by decreasing insulin clearance. Surprisingly, such effect was not noted after sinigrin administration. However, it is known that the hydrolysis of sinigrin leads to the formation, apart from allyl

isothiocyanates, also thiocyanides and nitriles, the latter of which are the most toxic hydrolysis products of glucosinolates in natural environments [2]. Those degradation products could attenuate the AITC action on insulin elevation. Therefore, the influence of additional sinigrin degradation products could also mitigate significantly the enhancement of liver glycogen content, which was observed after gavage of two doses of AITC, which stimulates glucose uptake indirectly by increased insulin secretion from pancreas. Despite this action, surprisingly, the blood serum glucose level was unchanged. This phenomenon could be explained, once again, by the observed exceptionally high FFA levels in this research. As indicated by other researchers [22], elevated serum FFA increase hepatic gluconeogenesis as well as reduce the ability of insulin to suppress this process in the liver, balancing the serum glucose concentration.

To conclude, the results of this study demonstrate that AITC is an active derivative of SIN, and the biological activity of the latter depends on the time that related endogenous processes degrade sinigrin to corresponding allyl isothiocyanate. The metabolic effect of AITC is multidirectional, indicating its impact on many organs like liver as well as pancreas, intestine *in vivo* action and rat adipocytes *in vitro*. Unfortunately, its influence is adverse for the majority of metabolic pathways of carbohydrates and lipids in rat model and potentiated with the time of its action. AITC, independently of dose and time, disturbed liver homeostasis. Elongated time of its activity changes the end-product in liver served as energy storage from triacylglycerols into glycogen. Short AITC action, except for steatosis, enhanced glycaemia (at higher dose). Long AITC treatment intensified liver glycogen store as a consequence of noted insulinaemia (action on pancreas), and with SIN evoked cholesterolaemia (action on intestine) and serum FFA increase (action on fat cells). Beneficial sides of their activity concerned triacylglycerols drop in the blood serum. AITC can augment basal and epinephrine-induced lipolysis; however, the stimulation of basal lipolysis and the AITC influence on epinephrine action seems to be dependent on many factors, certainly, on its dose. It would be urged that any further increase (abuse?) in dietary SIN and AITC should be also preceded by thorough and comprehensive investigations of their metabolic action.

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