

Acute sulforaphane action exhibits hormonal and metabolic activities in the rat: *in vivo* and *in vitro* studies

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ABSTRACT: So far, only the chronic effect of sulforaphane (SF) on metabolism was examined. This study sheds more light on SF potential ability of regulating lipid, carbohydrate, and hormonal metabolism during its acute action in *in vivo* and *in vitro* conditions. In the *in vivo* trial, rats were given once intragastrically 10 or 20 mg/kg of SF and were decapitated 4 h after the single intragastric treatment. The serum and the liver were collected to assay lipid, carbohydrate, and hormonal parameters. Additionally, we evaluated the acute direct *in vitro* action of SF (1.5 h) on basal and insulin-stimulated lipogenesis and basal and epinephrine-induced lipolysis in isolated primary rat adipocytes at 1 μ M, 10 μ M, and 100 μ M concentrations. The SF hormonal action was dose-dependent. In the *in vivo* trial, the higher dose evoked a significant insulin release ($P \leq 0.01$) and showed a tendency to limit the secretion of leptin from adipocytes compared with the control animals. Surprisingly, two applied SF doses did not cause any changes in serum glucose level and liver glycogen content. Both SF doses reduced HDL- and increased LDL-cholesterol level ($P \leq 0.05$), evoked a drop of liver triacylglycerol content ($P \leq 0.05$) compared with the control rats. In the *in vitro* study, only 100 μ M SF evoked elevation of basal- and epinephrine-induced lipolysis and inhibition of basal- and insulin-induced lipogenesis in comparison with the control ($P \leq 0.001$). SF adipocyte influence was independent of epinephrine and insulin action. Recapitulating, SF exhibited a tendency towards limiting lipid synthesis in adipocytes as well as in the liver, possibly via Nrf2 pathway. The disturbance in the LDL- to HDL-cholesterol ratio and dose-dependent increase in insulin concentration at normal glycaemia were connected probably with the SF capability to generate temporarily ROS in the pancreas and in the vascular endothelial cells in *in vivo* trials.

Keywords: broccoli; hormones; lipid metabolism; carbohydrate metabolism

INTRODUCTION

Sulforaphane (SF) belongs to the isothiocyanate class and it is found abundantly in many cruciferous vegetables. Especially broccoli sprouts are very rich in glucosinolate glucoraphanin, which is converted to SF in the gastrointestinal system of mammals during macerating or chewing (mastication) of raw vegetables. According to Shapiro et al. (2006), glucoraphanin represents 75% of all glucosinolates in the sprouts. Interestingly, broccoli sprouts differ from mature broccoli plants in

a higher content of glucoraphanin even up to 50 times on a gram fresh weight basis (Shapiro et al. 2001). The proper daily intake or an ideal dosage of sulforaphane is not known. However, typical recommendations range from 200 to 400 mg daily. Supplementation of 0.1–0.5 mg/kg SF to rats has been noted to be bioactive (<https://examine.com/supplements/sulforaphane/>). Isothiocyanates are approximately six times more bioavailable than glucosinolates (Rodriguez-Cantu et al. 2011). The pharmacokinetics of SF was assessed after oral dose of 50 μ mol SF (10 mg) in male Fisher F344 rat

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weighing 135 g. SF at the given dose (74 mg/kg BW) displayed a fairly rapid absorption. The availability of SF in plasma was very fast. The SF plasma concentration occurred at 1 h and its peak (20.8 µM) at 4 h and declined with a half-life of 2.23 h (Hu et al. 2004). On the basis of the above information and other available pharmacokinetics data (Ye et al. 2002; Cornblatt et al. 2007; Li et al. 2013), two SF doses (10 mg/kg and 20 mg/kg) were used in the *in vivo* trial. It means, that approximately 0.06–0.12 g raw material (seeds of young sprouts broccoli) had to be consumed by 200 g rats to produce SF at the doses of 10–20 mg/kg BW. In the *in vitro* experiment, SF concentrations ranged from 1 to 100 µM and the incubation time was set at 1.5 h.

Among numerous natural products of plant origin, SF has demonstrated promising potentials. In early research, it was mainly linked to anticarcinogenic and anti-proliferative activities and scavenging of reactive oxygen species (ROS) capacity (Okulicz et al. 2010). SF has been shown to be effective in blocking initiation as well as progression of various chemically induced carcinogenesis in animals. On the other hand, there is still limited information regarding SF as a nutraceutical source for the protection or management of pandemic levels of worldwide diseases like diabetes and hypertension by its influence on carbohydrate, lipid homeostasis in organs like pancreas, liver, adipocyte tissue. Some recent studies in clinical, animal, and *in vitro* models suggested SF antidiabetic properties (Choi et al. 2012, 2014; De Souza et al. 2012; Lee et al. 2012; Bahadoran et al. 2013) as well as cardiovascular and antihypertensive protection (Shan et al. 2010; Bahadoran et al. 2012a; Senanayake et al. 2012; Bai et al. 2013). Potential efficacy of SF makes it even a good choice for supplementary treatment in type 2 diabetes for normalization of changes in blood glucose and insulin sensitivity (Bahadoran et al. 2012b, 2013). However, the knowledge about SF influence on metabolism has been related only to its long-term action. SF has been shown to elicit a reduction of plasma total cholesterol, LDL-cholesterol, triacylglycerols (TG), and an increase in HDL-cholesterol in numerous long-term clinical and animal studies (1–7 weeks treatments) (Muraşhima et al. 2004; Lee et al. 2009; Rodriguez-Cantu et al. 2011; Bahadoran et al. 2012a). In our laboratory, long-running oral administration of SF (10 mg/kg BW, i.e. the same dose used in the

presented study), given as a single morning dose for two weeks, also showed a clear influence on lipid metabolism in Wistar rats (increased HDL-cholesterol, drop in TG concentrations in the blood serum) (Okulicz et al. 2010). Moreover, a 4-week consumption of 10 g/day broccoli sprouts powder resulted in a marked decrease in serum insulin concentration in a clinical trial (Bahadoran et al. 2012b). Also *in vitro* trials showed that prolonged exposure to SF (24 h) limited basal insulin secretion. In addition, insulin secretion stimulated by 20 mM glucose was significantly attenuated (Fu et al. 2013). Because of the lack of information about the SF acute influence on organisms an attempt was made to fill in this gap.

MATERIAL AND METHODS

There was no sign of liver damage (hepatotoxicity) and any cytotoxic effect after oral administration of SF at two experimental doses of 10 mg/kg BW and 20 mg/kg BW (AspAT and ALAT in normal activity).

Protocols of experiments were reviewed and approved by the local ethical commission for investigations on animals.

SF was purchased from ICN Biomedicals Inc. (Aurora, USA). All other chemicals were purchased from Sigma Chemical (St. Louis, USA).

In vivo experiment

Animal maintenance. The research was conducted on 24 growing male Wistar rats with the initial BW of 120 ± 5 g (7–8 weeks of age). Male Wistar rats were obtained from Brwinow (Poland). The animals were maintained under standard conditions of light (12 h) and temperature ($22 \pm 2^\circ\text{C}$) with access to laboratory pellets and tap water *ad libitum*. The rats were fed a complete laboratory diet (Labofeed, Poland) containing: crude protein 17%, crude fat 3.5%, crude fibre 7%, and 12.15 MJ/kg net energy. The animals were randomly divided into three groups consisting of eight rats each. They were allowed to acclimatize for at least 7 days before the commencement of the study. The experimental groups of rats were given once intragastrically 10 mg/kg BW or 20 mg/kg BW SF, respectively. These doses of SF were prepared as a solution by dissolving SF in 0.85% NaCl and were given at 5 ml/kg BW. Animals from control groups received an equivalent volume of the vehicle

0.85% NaCl. Animals were decapitated 4 h after the single intragastric treatment. Blood samples and liver samples were collected and stored at -80°C .

Measurement of hormonal and lipid parameters. The serum was used for the determination of blood hormones: insulin, leptin, adiponectin, and other blood parameters: AspAT, ALAT, glucose, free fatty acids (FFA), TG, total, free, esterified cholesterol (CHL), and total cholesterol in high-density lipoproteins (HDL) as well as low-density lipoproteins (LDL). Additionally cholesterol, TG, and glycogen were determined in the liver. Levels of hormones were determined in the blood serum using commercial assay kits, specifically: insulin, leptin, adiponectin using an immunoradiometric kit specific for rat hormone (Linco Research, St. Charles, USA).

FFA were determined according to Duncombe (1964) and TG were assayed by the method of Foster and Dunn (1973). Total, free, and esterified cholesterol levels were measured by the enzymatic method of Richmond (1973). Liver cholesterol and TG were determined similarly as in the serum after extraction of total lipids using the method of Folch et al. (1975). The obtained extracts were evaporated before use for cholesterol determination. Glucose was colorimetrically assayed by the enzymatic method of Hugget and Nixon (1957) using glucose oxidase, peroxidase, and o-dianisidine. The amount of the liver glycogen was determined using glucose method after extraction in 30% KOH and hydrolysis with amyloglucosidase.

Cholesterol in high density lipoprotein (HDL), in low density lipoprotein (LDL), AspAT, and ALAT in the serum were measured using the enzymatic kit from Pointe Scientific Inc. (Lincoln Park, USA).

***In vitro* experiment**

Preparation of adipocytes. After 1 week of adaptation, ten 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 (1963) with minor modifications by Szkudelska et al. (2000). 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 mmol/l NaCl, 4.8 mmol/l KCl, 1.3 mmol/l CaCl_2 , 1.2 mmol/l KH_2PO_4 , 1.2 mmol/l MgSO_4 , 24.8 mmol/l NaHCO_3) with 3mM glucose, 3% bovine serum albumin (BSA, fraction V), 10 mmol/l

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 four times with warm (37°C) Krebs-Ringer buffer without collagenase, filtered through nylon mesh, and counted under the microscope LSM 510 (Carl Zeiss, Jena, Germany) with a Bürker-Türk counting chamber. Cells viability was about 95% as determined by trypan blue exclusion.

Lipogenesis. Adipocytes (10^6 cells/ml) were incubated in plastic tubes at 37°C with Krebs-Ringer buffer, pH 7.4, containing 3% BSA, 10 mmol/l HEPES, 0.5 μCi of [$U\text{-}^{14}\text{C}$]glucose (specific activity 9.80 GBq/mmol, New England Nuclear Research Products), 5.56 mmol/l unlabelled glucose in the absence or presence of 1 nmol/l insulin. Similarly as in the case of lipolysis, SF was dissolved in DMSO and was added to the buffer with adipocytes to the final volume of 1 ml. The final concentrations of SF were 1 $\mu\text{mol/l}$, 10 $\mu\text{mol/l}$, and 100 $\mu\text{mol/l}$. Each experiment was performed three times with five replicates. Incubations were carried out with shaking for 90 min at 37°C . The reaction was stopped by adding 5 ml of Dole's extraction, containing isopropanol-heptane-1 N H_2SO_4 (40 : 10 : 1). Tubes were shaken and then 2 ml of H_2O and 3 ml of heptane were added for lipid extraction. After shaking, samples of the upper phase were transferred into counting vials containing a scintillation cocktail OptiPhase HiSafe 3 (PerkinElmer, Waltham, USA) and total lipid radioactivity was determined.

Lipolysis (adipolysis). Fat cell suspensions (10^6 cells per ml) were incubated in plastic tubes at 37°C for 90 min with the Krebs-Ringer buffer containing 3 mmol/l glucose, 10 mmol/l HEPES, and 3% BSA in the absence or the presence of 1 $\mu\text{mol/l}$ epinephrine. The examined SF was dissolved in DMSO and 10 μl of this solution (or DMSO alone in the case of control tubes) were added to 990 μl of the buffer with adipocytes. The final concentrations of SF were 1 $\mu\text{mol/l}$, 10 $\mu\text{mol/l}$, and 100 $\mu\text{mol/l}$. 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 (1973).

Statistical analysis. The obtained results from the *in vivo* experiment were averaged per group for each parameter and SEM was calculated. The statistical comparison of data was done by one-

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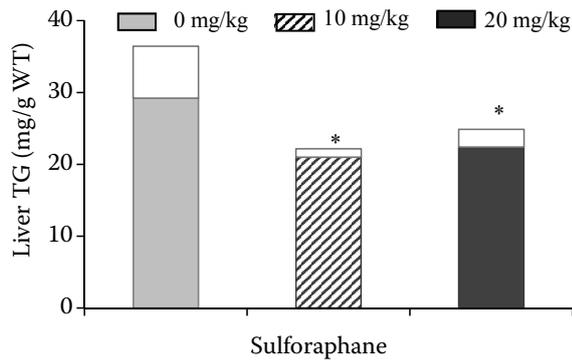


Figure 1. Effect of a 4-hour action of sulforaphane at two doses on triacylglycerols (TG) content in the liver. Values are given as means ± SEM for eight animals

WT = wet tissue

* $P \leq 0.05$, means differ significantly from the control value

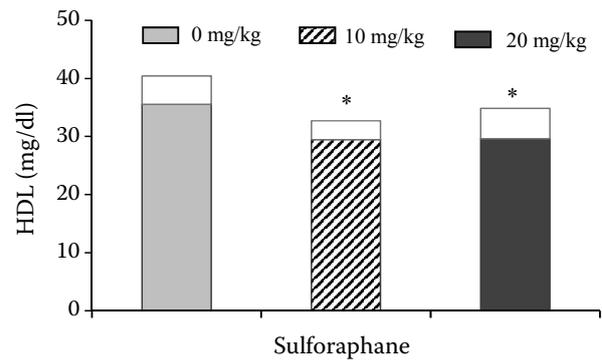


Figure 2. Effect of a 4-hour action of sulforaphane at two doses on HDL-cholesterol concentration. Values are given as means ± SEM for eight animals

* $P \leq 0.05$, means differ significantly from the control value

way ANOVA followed by the Duncan’s Multiple Range Test at $P \leq 0.05$ and $P \leq 0.01$.

The means ± SEM from three independent experiments in five replications of each experiment performed *in vitro* were evaluated statistically using the analysis of variance and Duncan’s Multiple Range Test. Differences were considered significant at $P \leq 0.001$.

RESULTS

Effects of SF on lipid, carbohydrate, and hormonal parameters in *in vivo* trial. SF at both doses was not found to change serum lipid parameters: FFA, TG, total, free, and esterified cholesterol (Ta-

ble 1). However, SF at both doses significantly ($P \leq 0.05$) limited TG formation in the liver (Figure 1) without any influence on liver cholesterol (Table 1). Apart from the absence of changes in the serum total cholesterol concentration, SF at both doses strongly disturbed the proportion of LDL/HDL-cholesterol. LDL-cholesterol concentration was found significantly higher (Figure 3), while HDL-cholesterol decreased considerably ($P \leq 0.05$) (Figure 2).

The higher SF concentration (20 mg/kg) strongly ($P \leq 0.01$) affected insulin release from pancreas (Figure 4). SF did not alter the serum adiponectin concentration and carbohydrate parameters like serum blood glucose level and liver glycogen content (Table 1).

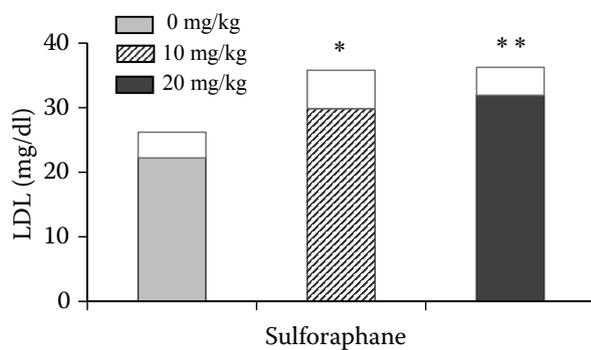


Figure 3. Effect of a 4-hour action of sulforaphane at two doses on LDL-cholesterol concentration. Values are given as means ± SEM for eight animals

* $P \leq 0.05$, ** $P \leq 0.01$, means differ significantly from the control value

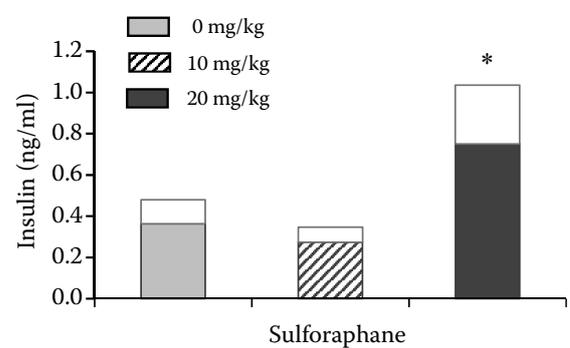


Figure 4. Effect of a 4-hour action of sulforaphane at two doses on insulin concentration. Values are given as means ± SEM for eight animals

* $P \leq 0.01$, mean differs significantly from the control value

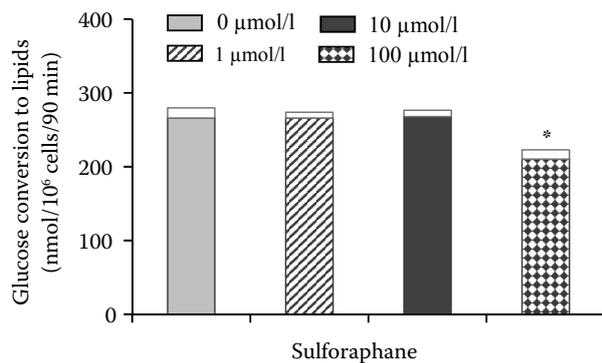


Figure 5. Effect of sulforaphane on basal lipogenesis from glucose in isolated rat adipocytes. Values represent means \pm SEM of 15 determinations from three separate experiments

* $P \leq 0.001$, mean differs significantly from the control value

The blood leptin levels were slightly lower after administration of the higher dose of SF (17.7%) compared with the control animals (Table 1).

The blood AspAT and ALAT were found at normal activity.

Effect of different doses of SF on basal lipogenesis and insulin-stimulated lipogenesis (1 μmol/l) in in vitro experiment. SF influenced lipogenesis in a dose-dependent manner. Only the highest dose of SF (100 μmol/l) inhibited basal lipogenesis by 20.9% as well as insulin-stimulated lipogenesis (31.9%) at $P \leq 0.001$. SF administered

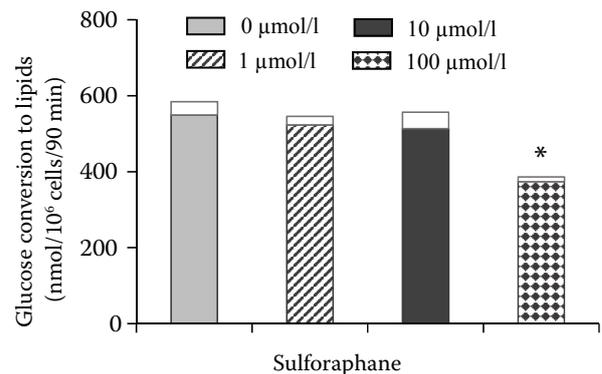


Figure 6. Effect of sulforaphane on insulin-stimulated lipogenesis from glucose in isolated rat adipocytes. Values represent means \pm SEM of 15 determinations from three separate experiments

* $P \leq 0.001$, mean differs significantly from the control value

at two lower doses (1 and 10 μmol/l) did not evoke any lipogenic changes (Figures 5, 6).

Effect of different doses of SF on basal lipolysis and epinephrine-stimulated lipolysis (1 μmol/l) in in vitro experiment. SF failed to exert changes in basal as well as epinephrine-induced lipolysis at 1 μmol/l and 10 μmol/l. However, the highest concentration of SF (100 μmol/l) elevated significantly basal lipolysis (41.9%) as well as epinephrine stimulated lipolysis (18.7%) in comparison with the control at $P \leq 0.001$ (Figures 7, 8).

Table 1. Statistically unchanged hormonal, enzymatic, lipid, and carbohydrate parameters after a 4-hour sulforaphane (SF) action at two doses (10 mg/kg BW and 20 mg/kg BW) in rats

Parameters in blood serum and liver	Control	SF 10 mg/kg	SF 20 mg/kg
Leptin (ng/ml)	3.67 \pm 0.42	3.58 \pm 0.57	3.02 \pm 0.28
Adiponectin (μg/ml)	6.19 \pm 0.67	5.98 \pm 0.56	6.29 \pm 0.58
AspAT (IU/l)	49.84 \pm 2.10	50.39 \pm 5.32	48.51 \pm 3.18
ALAT (IU/l)	7.51 \pm 0.97	6.74 \pm 1.04	7.44 \pm 2.92
Glucose (mmol/l)	5.15 \pm 0.14	5.24 \pm 0.15	4.86 \pm 0.07
Free fatty acids (mmol/l)	0.14 \pm 0.007	0.12 \pm 0.002	0.12 \pm 0.007
Triacylglycerols (mmol/l)	1.98 \pm 0.25	2.09 \pm 0.20	1.79 \pm 0.06
Total cholesterol (mmol/l)	1.47 \pm 0.15	1.38 \pm 0.14	1.64 \pm 0.16
Free cholesterol (mmol/l)	0.52 \pm 0.07	0.48 \pm 0.07	0.59 \pm 0.06
Esterified cholesterol (mmol/l)	0.94 \pm 0.10	0.90 \pm 0.10	1.18 \pm 0.08
Liver cholesterol (mg/g WT)	1.89 \pm 0.14	2.00 \pm 0.14	2.23 \pm 0.09
Liver glycogen (mg/g WT)	38.08 \pm 3.20	36.51 \pm 2.25	33.81 \pm 1.86

BW = body weight, WT = wet tissue
values are given as means \pm SEM for eight animals

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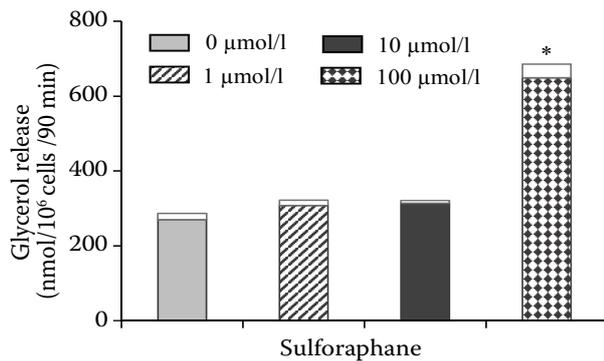


Figure 7. Effect of sulforaphane on basal lipolysis in isolated rat adipocytes. Values represent means \pm SEM of 15 determinations from three separate experiments

* $P < 0.001$, mean differs significantly from the control value

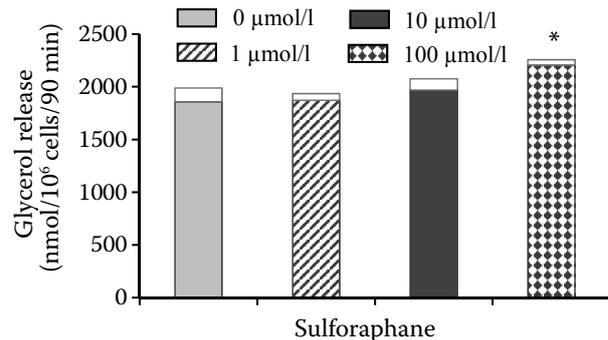


Figure 8. Effect of sulforaphane on epinephrine-stimulated lipolysis in isolated rat adipocytes. Values represent means \pm SEM of 15 determinations from three separate experiments

* $P < 0.001$, mean differs significantly from the control value.

DISCUSSION

Effect of SF on lipid and carbohydrate metabolism in in vivo conditions. The obtained metabolic results after acute SF action confirmed its fast absorption and bioavailability (consistent with previous data), but these changes showed some contradictions with respect to recent results concerning SF long-term metabolic and hormonal effects.

SF was reported to exert a favourable influence on cholesterol metabolism. Improvement of cholesterol metabolism after a one-week intake of broccoli sprouts in humans was proved first by Murashima et al. (2004). A clinical study conducted with 12 healthy individuals that consumed 100 g broccoli sprouts per day for 1 week showed a reduction in plasma total and LDL-cholesterol in males and an increase in HDL-cholesterol in females. Recently, Bahadoran et al. (2012 a) reported that taking a broccoli sprout powder at the dose of 10 g per day for 4 weeks in a clinical trial significantly decreased oxidized LDL/LDL-cholesterol ratio, serum TG, and simultaneously considerably increased HDL-cholesterol concentration. In our previous chronic experiment, the same trend of lipid changes in the serum of male Wistar rats was observed. Daily in the morning the animals were given a single dose of SF (10 mg SF/kg BW) for two weeks. Such treatment exerted a clear influence on lipid metabolism; HDL-cholesterol increased (29%) and serum TG concentrations dropped down (13%) (Okulicz et al. 2010). Lee et al. (2009) also confirmed a reduction in plasma total

cholesterol, LDL-cholesterol, TG, and an increase in HDL-cholesterol in male Sprague-Dawley rats after a 4-week consumption of 400 mg/kg BW ethanolic extract from broccoli sprouts. Because all the above presented results were obtained after chronic SF treatment, the unexpected lack of changes in total cholesterol in the present trial (Table 1) seems to be due to a limited time of SF action. The lowering of cholesterol levels reported in long-term experiments was attributed to the absorption site competition (isothiocyanates bind with bile acids reducing fat absorption), reduced synthesis of endogenous cholesterol, as well as to the increase in coprostanol excretion (Kahlon et al. 2007; Rodriguez-Cantu et al. 2011). Additionally, according to these researchers, the mechanism of anticholesterolemic effects of SF also might have involved its influence on genetic expression of some proteins taking part in the regulation of lipid homeostasis: sterol regulatory element binding protein (SREBP), HMG-CoA reductase (HMGP), fatty acids synthase (FAS), LDL receptors (LDLR), apolipoprotein B (ApoB100) (Rodriguez-Cantu et al. 2011). The most surprising and dissimilar to foregoing results was not only the lack of anticholesterolemic properties of SF. We also noticed a significant disturbance in HDL/LDL-cholesterol proportion after the short SF action (Figures 2, 3). So far, SF was believed to increase HDL-cholesterol fraction, among others by the inhibition of endothelial lipase (EL) expression (Kivela et al. 2010). This endothelial enzyme was shown to be directly upregulated by inflammation. The EL overexpression during inflammation

and/or by inflammatory cytokines decreases HDL concentration. Generally, SF is recognized as an anti-inflammatory agent in pathological hyperglycemic and oxidative conditions as well as an inducer of phase 2 enzymes via the nuclear factor E2-related factor 2 (Nrf2) transcriptional activator (Shan et al. 2010; Bahadoran et al. 2013). Recent documentation shows that the exposure to SF can also result in a transient ROS burst, the duration and magnitude of which are both dependent on the SF concentration and exposure period (Ferreira de Oliveira et al. 2014). The occurrence of oxidative stress reliant on the dose of isothiocyanates and its short-time action was recorded in our previous study (Okulicz et al. 2005). SF evoked inflammation at normal glycaemia by a transient production of ROS seems to be highly possible also in the presented short-term study. It is quite likely that the Nrf2 activation in vascular endothelial cells did not finish under the given experimental conditions and that the antioxidant response was limited. This seems to be additionally strengthened by the glutathione depletion. Conjugation of SF with glutathione (a necessary step in SF metabolism) depletes its intracellular concentration and probably lowers the oxidative stress threshold of the cells (Ferreira de Oliveira et al. 2014).

The key role in the regulation of energy homeostasis leading to alteration of plasma TG in chylomicrons, VLDL particles and steatosis in the liver is played by peroxisome proliferator-activated receptors (PPARs) (Gavrilova et al. 2003; Bahadoran et al. 2013). There is evidence that SF has the potential to modulate PPARs (Hu et al. 2006). PPAR γ up-regulation has been linked to exacerbated steatosis by the mechanism involving the activation of lipogenic genes and *de novo* lipogenesis and increased hepatic TG (Moran-Salvador et al. 2011). Recently, down-regulation of PPAR γ by SF in adipocytes was confirmed by Choi et al. (2012, 2014). In our trial, the TG levels in blood serum were not altered, however, the TG content in the liver was decreased after the administration of both SF doses (Figure 1). Therefore, down-regulation of PPAR γ in the liver by SF was highly possible in our experiment. This supposition may be confirmed by the additional SF capacity to induce the above-mentioned Nrf2 pathway (Bahadoran et al. 2013). Nrf2 activation also directly targets lipogenic gene expression such as PPAR γ and, thereafter, modulates hepatic

lipid homeostasis and suppresses lipid synthesis (Huang et al. 2010).

De Souza et al. (2012) reported that some phytochemicals *per se* are capable of preventing the conversion of glucose-6-phosphate to glucose by the inhibition of glucose-6-phosphatase, preserving the glycogen level. However, our short SF action did not show any changes in the liver glycogen content suggesting the lack of acute SF influence on carbohydrate metabolism in hepatocytes as well as on glucose in blood serum (Table 1).

Effect of SF on hormonal status of pancreas and adipocytes. SF was also found to influence the hormonal status. In clinical studies, Bahadoran et al. (2012b) showed that supplementation of patients' diets with 10 g/day broccoli sprout powder for 4 weeks resulted in a decrease in their serum blood insulin. This status was coherent with the antioxidative and/or anti-inflammatory properties of SF in broccoli sprouts (Bahadoran et al. 2012b). This is inconsistent with the results obtained in the presented trial. The applied higher dose of SF (20 mg/kg BW) caused a robust insulin increase (76%) after 4 h (Figure 4). Because SF stimulated a significant insulin secretion without any rise in blood glucose concentration, some direct SF influence on pancreas could be suggested. The ability of SF to promote ROS generation in several cell types was demonstrated by Ferreira de Oliveira et al. (2014). Lately, the activation of insulin secretion by transient increase in ROS immediately following the onset of SF was proved by Fu et al. (2013). Therefore, it is highly probable that also in our experiment insulin was secreted in a similar manner. Furthermore, according to Fu et al. (2013) and Souza et al. (2013) the secreted insulin accumulates to higher levels following SF stimulation in a time- and concentration-dependent manner in the presence of fairly low glucose. Such a dose-dependent increase in insulin concentration at normal glycaemia was also proved in our trial, where only a higher dose (20 mg/kg) caused insulin secretion.

The adipose tissue secretes adipogenic regulatory proteins (adipokines) such as leptin and adiponectin. Leptin production is positively correlated with obesity, while adiponectin production is inversely related to it (Choi et al. 2014). Choi et al. (2014) showed that SF in the high fat diet (HFD) rats significantly attenuated the induced increases in leptin expression and increased the HFD-induced reduction in adiponectin expression. In our

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healthy rats, in the *in vivo* trial, adiponectin was unchanged. However, we noticed a slight downslide in leptin concentration, especially at higher SF dose (Table 1). Such trend of change is consistent with the observations of other researchers. It is highly probable that SF exerted some impact on cellular lipid metabolism and total energy expenditure via the above-mentioned influence on Nrf2 activation. Nrf2 is recognized as a negative regulator of lipid synthesis (Vomhof-DeKrey et al. 2012). Therefore, adipocyte hypertrophy reduction in the presence of SF could have caused the observed slight downslide in leptin concentration. In an attempt to recognize a direct SF influence on adipocytes, we extended our trial also by the *in vitro* experiment.

Effect of SF on lipogenesis and lipolysis in the adipocyte tissue in *in vitro* conditions. The *in vivo* peak concentration of 20 µM SF after 50 µmol oral administration of SF (58–73 mg/kg BW) offers a clear relevance for numerous *in vitro* cell culture studies, where the range of 1–30 µM SF was typically used (Choi et al. 2012; Lee et al. 2012). According to Choi et al. (2012), a 6-day SF action decreased the accumulation of lipid droplets and inhibited the elevation of TG in the 3T3-L1 cells by the half-maximal inhibitory concentration 7.3 µM. In our *in vitro* study, a short SF treatment (1.5 h) showed an inhibitory effect on basal- and insulin-stimulated lipogenesis only at the highest concentration (100 µM) in isolated primary rat adipocytes (Figures 5, 6). SF at two lower doses remained indifferent to lipogenic action, presumably owing to limited time treatment.

According to Lee et al. (2012), a 24 h treatment of SF appeared to be able to increase the rate of glycerol release into the medium from mature adipocytes (obtained from 3T3-L1) in a concentration-dependent manner up to 10 µM via the stimulation of hormone sensitive lipase phosphorylation at Ser-563 and the inhibition of AMPK phosphorylation. The lipolysis activity of SF was also confirmed in our *in vitro* trial. However only 100 µM of SF was active enhancing the basal as well as epinephrine stimulated lipolysis (Figures 7, 8). SF at this dose showed an additive influence on catecholamine activity.

Based on these observations, certainly, SF was not a neutral compound for fat cells. Only a very high SF concentration was capable of interfering with lipid metabolism at acute treatment. Its adipocyte influence was independent of epinephrine

and insulin action steps. The obtained discrepancies in all presented *in vitro* studies seem to be linked with the varying time of incubation with SF as well as the diversity of experimental cells and their different stage of development.

Recapitulating, in both *in vivo* and *in vitro* studies we did not explore the cellular redox homeostasis (balance) and energy metabolism caused by plausible changes in the transcription factor Nrf2. Further studies are necessary to understand the mechanism underlying the observed changes during SF acute action.

CONCLUSION

The obtained lipid as well as hormonal changes in rats after 4 h of SF action provided an extra proof of its rapid absorption and bioavailability at two normal dietary doses. Short SF treatment strongly affected lipid metabolism without influencing carbohydrate status. The *in vivo* and *in vitro* trials showed that SF was not a neutral compound for rat fat and liver cells. It is quite possible that SF limited lipid synthesis in adipocytes as well as in the liver (drop in the liver TG content) via the Nrf2 pathway. However, in the *in vitro* study, lipogenesis suppression and lipolysis activation were only noticeable at a very high SF concentration (100 µM) during its short-term action. SF adipocyte influence was independent of epinephrine and insulin action. It seems highly probable that the observed changes in lipid and hormonal status in the *in vivo* trial (disturbance in LDL/HDL-cholesterol ratio in favour of higher LDL-cholesterol concentration, dose-dependent increase in insulin concentration at normal glycaemia) were connected with SF capability to generate transiently ROS in the pancreas as well as in the vascular endothelial cells at its short action. This phenomenon requires further animal studies.

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