

## Effects of indole-3-carbinol on metabolic parameters and on lipogenesis and lipolysis in adipocytes

M. OKULICZ, I. HERTIG, J. CHICHŁOWSKA

Department of Animal Physiology and Biochemistry, Faculty of Animal Breeding and Biology, University of Life Sciences in Poznań, Poznań, Poland

**ABSTRACT:** Indole-3-carbinol (I3C) was found to have possible anticarcinogenic, antioxidant and anti-atherogenic effects on the organism. So far, its influence on metabolic pathways has been unknown. This work was the first attempt to determine the carbohydrate and lipid metabolism changes *in vivo* after administration of 150 mg/kg b.wt./day I3C to male rats. Additionally, the aim of this trial was to evaluate the direct effect of I3C on basal and hormone-induced lipogenesis and lipolysis in isolated rat adipocytes at concentrations 1, 10, 100  $\mu\text{M}$  *in vitro*. We can corroborate that adipocytes are susceptible to the direct action of I3C. The incubation of adipocytes with I3C at the three above-mentioned concentrations resulted in its influence on restriction of glucose entry into adipocytes in the basal as well as insulin-stimulated conditions. However, it was observed that I3C at these concentrations strongly intensified basic and epinephrine-stimulated lipolysis. I3C also has a significant influence on metabolism *in vivo*. Its administration to rats caused a significant increase in the content of triglycerides and a decrease in glycogen in the liver. The considerable augmentation of glucose, triglycerides, cholesterol in high-density lipoprotein and insulin with a concomitant decrease in FFA concentrations was noted in the blood serum. I3C did not alter phospholipids, total, free, esterified cholesterol in the serum and the liver cholesterol. The results obtained *in vivo* and *in vitro* indicate that the effect of I3C is adverse for the majority of metabolic parameters which were investigated. The most important finding in this study is the effect of I3C on liver steatosis and that the observed lower lipogenesis at higher lipolysis in fat cells may be involved in the mechanism.

**Keywords:** indole-3-carbinol; lipid metabolism; carbohydrate metabolism; rat

Indole-3-carbinol (I3C) is a major derivative of glucobrassicin (3-indolylmethyl glucosinolate), a plant product common to vegetables of the class Cruciferae (McDanell et al., 1986). I3C is both an anti-initiator and a promoter of carcinogenesis depending on the timing and dose of administration. It has been found to inhibit the development of tumours in forestomach, glandular stomach, mammary gland, prostate, uterus, tongue, and liver of rodents, as well as in the trout liver, when administered prior to or during carcinogen expo-

sure by gavage or in the diet (Kim et al., 1997). The consumption of I3C by humans and rodents can lead to marked increases in activities of cytochrome P-450-dependent monooxygenases and in a variety of phase II drug metabolizing enzymes like glutathione S-transferase (GST) resulting in the increased hepatic metabolic capacity towards chemical carcinogens (Bjeldanes et al., 1991). The induction of four different P450 forms (P450, 1A1, 1A2, 2B1 and 3A) was described in the liver of rats fed I3C for as short a period as 2 days (Wortelboer

---

Supported by the State Committee for Scientific Research of Poland (Grant No. 3PO6D 015 25).

et al., 1992; Manson et al., 1997). Activated P450 enzymes are involved in carcinogen activation, increasing the production of reactive oxygen species (ROS). The induction of CYP1A1 by indole-3-carbinol has been associated with an increase in oxidative DNA damage in a cell culture, which suggests that the induction can lead to a leak of oxygen radicals (Park et al., 1996). These properties of I3C are considered to contribute to the known ambiguous property (anti- and pro-oxidative) of this compound.

Early studies demonstrated that I3C was effective only when given orally and not when given i.p. or i.v. Namely, under low pH conditions in stomach, several condensation reactions of I3C occur which result in the formation of 3, 3'-diindolylmethane (DIM) and indolylcarbazole (ICZ). I3C is an active inducer of CYP 4501A1 increasing 2-hydroxylation of oestrogens. Having a sustained oestrogen modifying effect I3C is a good candidate for clinical trials in women at an increased risk of developing breast cancer (Brignall, 2001). In addition to these indirect effects as a result of altered oestrogen metabolism, indole-3-carbinol as well as DIM has been shown to have direct effects on apoptosis and cyclin D, resulting in blockage of the cell cycle (Bradlow et al., 1999). Because it facilitates the inactivation of oestrogen, it is possible that I3C might tend to promote osteoporosis in postmenopausal women and could interfere with oestrogen therapies (birth control pills and hormone replacement therapy).

The mechanism of I3C action in the anticarcinogenic activity has been intensively investigated, and now it is quite well understood on the contrary to its influence on carbohydrate and lipid metabolism. It is known that the biologically active products of degradation of glucosinolate precursors can have an influence on metabolism in rats (Okulicz et al., 2005). Therefore, the purpose of our experiments was to ascertain the effect of I3C at a dose of 150 mg/kg body weight/day on some metabolic parameters and blood insulin concentration under physiological conditions in normal rats. In addition, the aim of our work was to investigate the direct effect of I3C on lipogenesis and lipolysis in isolated rat adipocytes not caused by hormonal and metabolic changes. It is worth noting that the alterations in the whole body lipid metabolism result also, at least in great part, from changes in adipocytes (Szkudelska et al., 2002).

## MATERIAL AND METHODS

### *In vitro* experiment

#### Preparation of adipocytes

After 7 days of adaptation the male rats weighing  $160 \pm 5$  g were sacrificed by decapitation and the epididymal adipose tissue was taken. Adipocytes were isolated according to the method of Rodbell (1964) with minor modifications (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 (118mM NaCl, 4.8mM KCl, 1.3mM CaCl<sub>2</sub>, 1.2mM KH<sub>2</sub>PO<sub>4</sub>, 1.2mM MgSO<sub>4</sub>, 24.8mM NaHCO<sub>3</sub>) with 3mM glucose, 3% bovine serum albumin (BSA, fraction V), 10mM 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 the warm (37°C) Krebs-Ringer buffer without collagenase, filtered through nylon mesh and counted under the microscope with Bürker-Türk counting chamber. Cell 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 3mM glucose, 10mM HEPES and 3% BSA in the absence or the presence of 1 µM epinephrine. The examined I3C was dissolved in DMSO and 10 µl of this solution (or DMSO only in the case of control tubes) was added to 990 µl of the buffer with adipocytes. The final concentrations of I3C were 1 µM, 10 µM, 100 µM. Each experiment was performed three times with five replications. Glycerol released from adipocytes, reflecting the intensity of lipolysis, was measured using the method of Foster and Dunn (1973).

#### Lipogenesis

Adipocytes ( $10^6$  cells/ml) were incubated in plastic tubes at 37°C with Krebs-Ringer buffer, pH 7.4, containing 3% BSA, 10mM HEPES, 0.5 µCi of (U-<sup>14</sup>C)glucose (specific activity 9.80 GBq/mmol,

New England Nuclear Research Products), 5.56mM unlabelled glucose in the absence or presence of 1nM insulin. Similarly like in the case of lipolysis, I3C was dissolved in DMSO and was added to the buffer with adipocytes to the final volume 1 ml. The final concentrations of I3C were 1 $\mu$ M, 10 $\mu$ M, 100 $\mu$ M. Each experiment was performed three times with five replications. 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<sub>2</sub>SO<sub>4</sub> (40:10:1). Tubes were shaken and then 2 ml of H<sub>2</sub>O 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 'Hi Safe' Wallac) and total lipid radioactivity was determined.

### *In vivo* experiment

#### Feeding experiment

Rats were administered I3C at an estimated dose of 150 mg/kg b.wt./day. This dose had no effect on food consumption, rat weight, or general appearance and behaviour and was comparable to those used by other researchers (Bradlow et al., 1991; LeBlanc et al., 1994; Stresser et al., 1995).

Male Wistar rats, initially weighing 120  $\pm$  5 g, were used in the experiment. Rats were kept in cages in standard conditions with a constant temperature of 21  $\pm$  1°C, 12-h dark-light cycle, and were fed a laboratory diet *ad libitum* (Labofeed, Kcynia, Poland). They were randomly divided into two groups consisting of eight rats each. During the

experiment lasting 14 days, the rats in both groups drank tap water *ad libitum*. Rats in one group were treated with 150 mg/kg b.wt. I3C. This compound was dissolved in maize oil and was administered intragastrically once a day for 14 days at the volume of 0.5 ml/100 g b.wt. The rats from the control group received the same volume of sole maize oil as a vehicle. Rats were decapitated 12–14 h after the last intragastric treatment and their blood serum and liver samples were collected and stored at –80°C until analysis.

The serum was used for the determination of blood glucose, free fatty acids (FFA), phospholipids, triglycerides (TG), total, free and esterified cholesterol, total cholesterol in high-density lipoproteins (HDL) and insulin.

In the liver cholesterol, triglycerides and glycogen were determined.

Glucose was assayed colorimetrically by the enzymatic method with glucose oxidase, peroxidase and o-dianisidine (Hugget and Nixon, 1957). Free fatty acids were determined according to Duncombe (1964) and triglycerides were assayed by the method of Foster and Dunn (1973). Total, free, and esterified cholesterol levels and cholesterol in HDL were measured by the enzymatic method of Richmond (1973). High-density lipoproteins were separated from the blood serum using polyethylene glycol 6 000 according to Demacker et al. (1980). Phospholipids were determined enzymatically with a kit provided by BioMerieux (France).

Insulin was assayed radioimmunologically using the kit specific to rat hormone (Linco Research, St. Charles, Missouri, USA).

Liver cholesterol was assayed after extraction of lipids (Folch et al., 1975) and evaporation of the

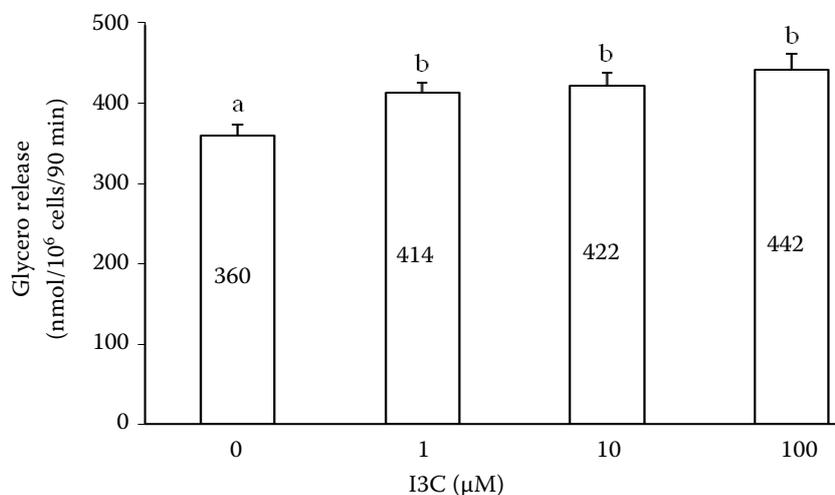


Figure 1. The effect of indole-3-carbinol on basal lipolysis in isolated rat adipocytes. Each column represents the mean  $\pm$  SEM for five replications ( $n = 5$ ). Mean values marked by different letter superscripts differ statistically ( $P \leq 0.05$ )

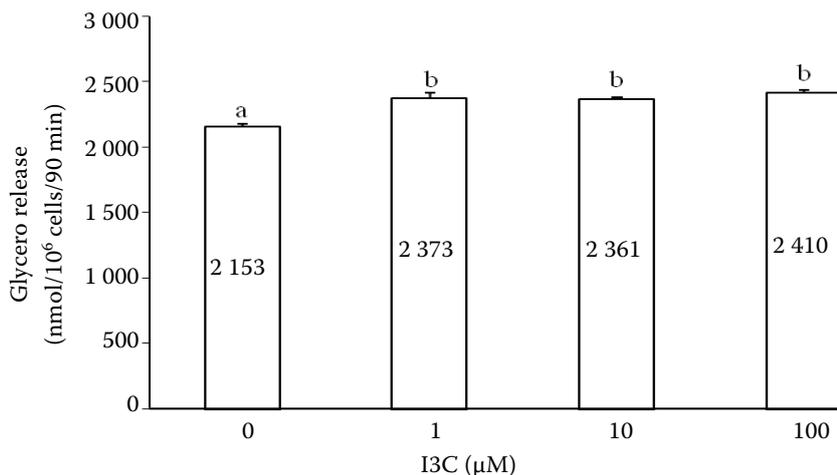


Figure 2. The effect of indole-3-carbinol on lipolysis stimulated by epinephrine (1 μM) in isolated rat adipocytes. Each column represents the mean ± SEM for five replications ( $n = 5$ ). Mean values marked by different letter superscripts differ statistically ( $P \leq 0.05$ )

extract (Richmond, 1973). The amount of liver glycogen was determined after its extraction in 30% KOH and hydrolysis with amyloglucosidase. Liver TG were assayed after extraction (Folch et al., 1975).

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

The experiment was performed according to rules accepted by the Local Ethical Commission for Investigations on Animals.

## RESULTS

### *In vitro*

#### Effect of I3C on lipolysis

I3C affected lipolysis significantly. Figures 1 and 2 show glycerol release from adipocytes exposed

to I3C (1 μM, 10 μM, 100 μM) in the absence and in the presence of epinephrine. I3C did not show a dose-dependent significant enhanced effect on basal lipolysis. We noted 15.0%, 17.2%, 22.8% increased basal lipolysis in comparison with the control, respectively (Figure 1). Epinephrine-stimulated lipolysis was also influenced by I3C at all concentrations. We noted 10.2%, 10.0%, 11.9% increased epinephrine-stimulated lipolysis (Figure 2).

#### Effect of I3C on lipogenesis

Figures 3 and 4 show ( $U\text{-}^{14}C$ )glucose conversion to lipids in adipocytes exposed to three doses of I3C (1 μM, 10 μM, 100 μM) in the absence and in the presence of insulin. We noted that I3C clearly inhibited basal lipogenesis at three different concentrations. The lipogenesis activity after the treatment with I3C in comparison with the control was 67.6%, 73.3% and 69.3%, respectively (Figure 3). Insulin-stimulated lipogenesis was also strongly reduced by I3C

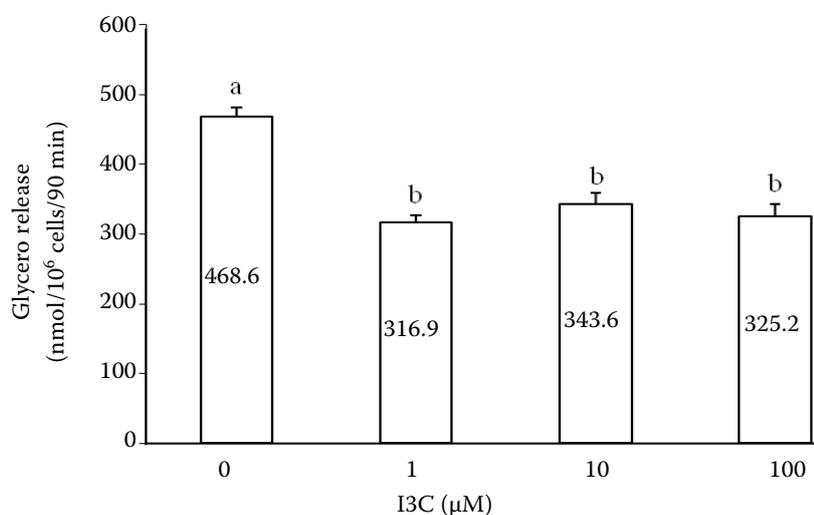


Figure 3. The effect of indole-3-carbinol on basal lipogenesis from glucose in isolated rat adipocytes. Each column represents the mean ± SEM for five replications ( $n = 5$ ). Mean values marked by different letter superscripts differ statistically ( $P \leq 0.05$ )

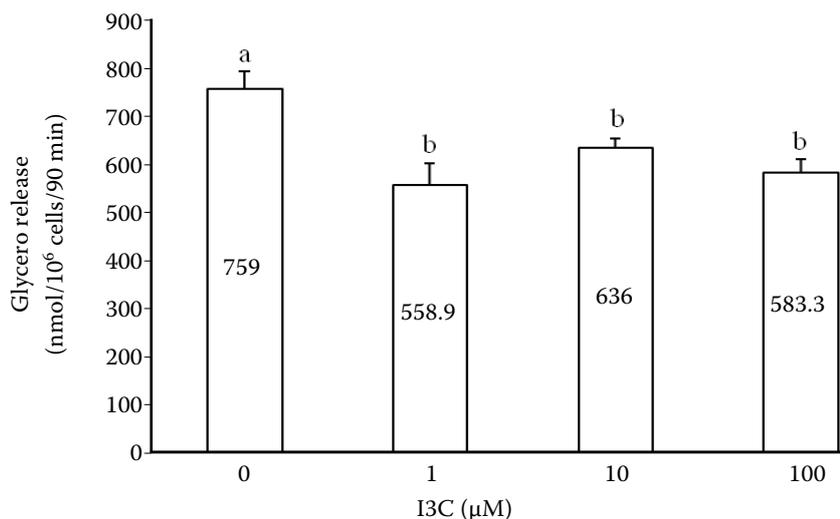


Figure 4. The effect of indole-3-carbinol on insulin-stimulated (1nM) lipogenesis from glucose in isolated rat adipocytes. Each column represents the mean  $\pm$  SEM for five replications ( $n = 5$ ). Mean values marked by different letter superscripts differ statistically ( $P \leq 0.05$ )

at all tested concentrations, in no dose-dependent manner. The insulin-stimulated lipogenesis activity was 73.6%, 83.8% and 76.9% in comparison with the control, respectively (Figure 4).

### *In vivo*

The results obtained in the experiment *in vivo* are presented in Table 1. The administration of

I3C at the dose of 150 mg/kg b.wt./day caused a significant increase in the content of triglycerides (60.9%) and a decrease in glycogen (23.8%) in the liver. A considerable increase in glucose (36.0%), triglycerides (32.2%), cholesterol in high-density lipoprotein (21.3%) and insulin (53.6%) with a concomitant decrease in FFA (65.4%) concentrations was noted in the serum.

I3C did not alter phospholipids, total, free, esterified cholesterol in the serum and liver cholesterol.

Table 1. The influence of oral administration of I3C on blood insulin and metabolic parameters in rats

Hormonal and metabolic parameters	Maize oil	Maize oil + I3C
<b>Blood serum</b>		
Insulin (ng/ml)	0.56 $\pm$ 0.07 <sup>a</sup>	0.86 $\pm$ 0.08 <sup>b</sup>
Glucose (mmol/l)	5.41 $\pm$ 0.22 <sup>a</sup>	7.36 $\pm$ 0.21 <sup>b</sup>
Free fatty acids (mmol/l)	0.26 $\pm$ 0.02 <sup>a</sup>	0.09 $\pm$ 0.01 <sup>b</sup>
Phospholipids (mmol/l)	1.21 $\pm$ 0.17	1.36 $\pm$ 0.15
Triglycerides (mmol/l)	2.70 $\pm$ 0.11 <sup>a</sup>	3.57 $\pm$ 0.11 <sup>b</sup>
Total cholesterol (mmol/l)	1.43 $\pm$ 0.07	1.48 $\pm$ 0.11
Free cholesterol (mmol/l)	0.49 $\pm$ 0.03	0.50 $\pm$ 0.03
Esterified cholesterol (mmol/l)	0.50 $\pm$ 0.05	0.49 $\pm$ 0.08
HDL-cholesterol (mmol/l)	0.47 $\pm$ 0.02 <sup>a</sup>	0.57 $\pm$ 0.04 <sup>b</sup>
<b>Liver</b>		
Cholesterol (mg/g WT)	2.12 $\pm$ 0.10	2.19 $\pm$ 0.09
Triglycerides (mg/g WT)	8.03 $\pm$ 0.65 <sup>a</sup>	12.92 $\pm$ 0.86 <sup>b</sup>
Glycogen (mg/g WT)	36.63 $\pm$ 1.78 <sup>a</sup>	27.92 $\pm$ 2.48 <sup>b</sup>

WT = wet tissue

I3C was administered intragastrically at the dose of 150 mg/kg body weight/day for 14 days. Values are given as mean  $\pm$  SEM for eight animals. Mean values in rows marked by different letter superscripts differ statistically ( $P \leq 0.05$ )

## DISCUSSION

Adipose tissue lipolysis is the major regulator of the body's supply of lipid energy because it controls the release of fatty acids into the plasma, where they circulate as FFA complexes to albumin. Hormone sensitive lipase of adipose tissue (HSL, EC 3.1.1.3) is the proximal and determinant enzyme for whole-body lipid fuel availability. We can corroborate that adipocytes are susceptible to the direct action of I3C (Figures 1, 2, 3, 4). It was demonstrated in the present report that I3C significantly induced basal as well as epinephrine-stimulated (1  $\mu$ M) lipolysis in isolated rat adipocytes at all used concentrations of I3C (1, 10, 100  $\mu$ M) (Figures 1, 2). The intensity of these processes was not significantly dependent on the I3C concentration. Basal lipolysis (triglyceride decomposition) is a hormone-independent process providing glycerol and fatty acids continuously. The ability of indole-3-carbinol to stimulate basal lipolysis indicates that the action of this compound involves changes in further steps of the lipolytic cascade, i.e. adenylate cyclase, cAMP content, protein kinase A (PKA), hormone sensitive lipase activity or translocation of this enzyme from the cytosol to the lipid droplet (Szkudelski and Szkudelska, 2002). The exact mechanism of I3C in this activation is difficult to ascertain at this stage. Whereas the noted intensified lipolysis in adipocytes in the presence of epinephrine indicates the stimulatory influence of I3C on the epinephrine action. The incubation of adipocytes with I3C at the three above-mentioned concentrations resulted in its influence on the restriction of glucose entry into adipocytes in the basal as well as insulin-stimulated conditions (Figures 3, 4). Glucose conversion to lipids is preceded by glucose transport into cells and involves its metabolism to acetyl-CoA and, finally, malonyl-CoA. Then, long-chain fatty acids are formed and esterified to triglycerides. The inhibitory effect of I3C on basal glucose metabolism to lipids indicates that it restricts predominantly insulin independent steps of lipogenesis, without influencing the insulin receptor. In basal lipogenesis glucose is transported predominantly via GLUT1 and this transport is insulin-independent. Therefore, it is possible that tested I3C attenuates lipogenesis via disturbance of the hexose transporter GLUT1. However, we noticed also the anti-lipogenetic action of I3C in insulin-stimulated conditions (Figure 4). It is quite possible that tested I3C, additionally, diminished the insulin-induced

uptake of glucose into adipocytes. I3C significantly attenuated glucose conversion to lipids, probably by disturbance of the function of insulin due to decreased glucose transporter GLUT4 translocation to the cell surface. The inhibitory effect of I3C on glucose transport may be accompanied by a substantial reduction in phosphatidylinositol 3-kinase (PI 3-K) activity. This enzyme regulates the insulin-induced translocation of glucose transporting protein-4 (GLUT4) and thereby enables the transport of glucose into fat cells (Szkudelska et al., 2007).

In the performed trial a significant influence of I3C on metabolism was also shown (Table 1). According to LeBlanc et al. (1994) the hepatic microsomal total cholesterol levels were not significantly altered following the treatment with 100 and 240 mg I3C/kg/day for 1 week in mice. It means that the pivotal activity of hepatic cholesterol 7 $\alpha$ -hydroxylase, which enhances cholesterol conversion to bile acids, and hepatic enzymes cholesterol ester hydrolase were not altered by I3C. Such a result was also obtained in our trial, at the used dosage of 150 mg/kg b.wt./day of I3C in rats. However, according to these authors I3C decreased serum cholesterol levels, which we did not observe in our experiment. The unchanged level of total, free, esterified cholesterol in the serum in our trial could suggest limited generation of acid condensation products from the used I3C dosage because the results of many studies demonstrated that not I3C per se, but several acid condensation products of I3C inhibit acyl-CoA:cholesterol acyltransferase (ACAT) activity at micromolar concentrations (Dunn and LeBlanc, 1994). It is worth underlining that the inhibition of intestinal/hepatic ACAT plays pivotal roles in the impediment of cholesterol uptake from the intestinal cells or accumulation in the liver.

I3C itself is found by many researchers to be a highly enzyme-inducing cytochrome. It is known that several drugs inducing the microsomal cytochrome P-450-dependent enzyme system in the liver and the intestine may increase plasma HDL concentrations (Nanjee et al., 1996). These authors do not exclude the possibility that some phytochemicals may also have such effect. Such hypothesis was confirmed in our trial, where I3C caused an increase in the plasma HDL concentration. Since in our experiment the drop in liver glycogen was accompanied by simultaneous augmentation in the blood insulin and glucose concentration, it

can be postulated that lower liver glycogen storage results from the rise of glycogenolysis in I3C treated rats. The high concentration of serum glucose could be elevated, additionally, by disturbance of hexose transporters GLUT1, GLUT4, which was confirmed in our *in vitro* experiment. Therefore, the indirect I3C effect on an increase in the insulin level in blood serum seems to be more likely, by its enhancing glycogenolysis and disturbance of hexose transporters GLUT1 and GLUT4.

The symbiotic relationship between adipose tissue and hepatic TG mobilisation is clearly disturbed by I3C in our research. This impediment was confirmed by the elevated concentration of TG in serum and in liver and lowered concentration of serum FFA *in vivo* with lower lipogenesis and higher lipolysis in adipocytes *in vitro*.

Under normal conditions *de novo* TG synthesis represents only 5% of liver TG, therefore the majority of liver TG is from serum or intestinal fatty acids. The serum FFA are derived mainly from adipose tissue. Fatty acids released by adipocytes are the major substrates for the hepatic very low-density lipoprotein (VLDL) production, and in turn, VLDL TG fatty acids are returned to adipose tissue by the action of lipoprotein lipase (LPL, EC 3.1.1.34) (Gibbons et al., 2000). The excess lipolysis may induce hypertriglyceridaemia via the increased production of very low-density lipoproteins (Coppack et al., 1994). Therefore the intensified lipolysis *in vitro* observed in our research may explain hypertriglyceridaemia in the liver *in vivo*. Because we observed the simultaneous liver and serum TG enhancement, it is highly probable that the observed TG increase in the serum was not from an excessive flux of VLDL out of liver but mainly from the influence of I3C per se on the decreasing clearance of triglyceride-rich lipoproteins in serum derived from liver and intestine. The enzyme responsible for the hydrolysis of triacylglycerols from plasma lipoproteins, mainly chylomicrons and very low-density lipoproteins, is the above-mentioned lipoprotein lipase. Its activity is influenced by the nutritional and hormonal status and by environmental conditions. Therefore the putative I3C inhibition action on LP seems to be very likely. This hypothesis could additionally be confirmed by FFA depletion in serum despite intensified lipolysis, which was observed *in vitro*. The FFA drop in serum resulted from the limited accumulation of TG stored within adipocyte cytosolic lipid droplets by the inhibition of FFA

release from VLDL TG. So I3C is hypothesized to attenuate not only lipogenesis *de novo* (by restriction of glucose entry into adipocytes) but also by inhibition of lipogenesis from FFA by LPL inactivation.

I3C showed multifaceted activities, influencing metabolic pathways during the long-term action *in vivo* and metabolism of isolated rat adipocytes *in vitro*. The elevated concentration of TG (in serum and liver), serum glucose, serum insulin and lowered concentration of serum FFA after I3C consumption show an adverse effect of this substance. HDL-cholesterol was moderately elevated after I3C administration, which is positive. To conclude, the obtained results indicate that the global effect of 150 mg/kg b.wt./day on lipid and carbohydrate metabolism is negative in rats.

## REFERENCES

- Bjeldanes L.F., Kim J.Y., Grose K.R., Bartholomew J.C., Bradfield C.A. (1991): Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol *in vitro* and *in vivo*: Comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proceedings of National Academy Sciences USA, 88, 9543–9547.
- Bradlow H.L., Michnovicz J., Telang N.T., Osborne M.P. (1991): Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. Carcinogenesis, 12, 1571–1574.
- Bradlow H.L., Sepcovic D.W., Telang N.T., Osborne M.P. (1999): Multifunctional aspects of the action of indole-3-carbinol as an antitumor agent. Annals of the New York Academy Sciences, 889, 204–213.
- Brignall M.S. (2001): Prevention and treatment of cancer with indole-3-carbinol. Alternative Medicine Review, 6, 580–589.
- Coppack S.W., Jensen M.D., Miles J.M. (1994): *In vivo* regulation of lipolysis in humans. *In vivo* regulation of lipolysis in humans. Journal of Lipid Research, 35, 177–193.
- Demacker P.N., Hijmans A.G., Vos-Janssen H.E., van't Laar A., Jansen A.P. (1980): A study of the use of polyethylene glycol in estimating cholesterol in high-density lipoprotein. Clinical Chemistry, 26, 1775–1779.
- Duncombe D. (1964): The colorimetric micro-determination of nonesterified fatty acids in plasma. Clinica Chimica Acta, 9, 122–125.
- Dunn S.E., LeBlanc G.A. (1994): Hypocholesterolemic properties of plant indoles. Inhibition of acyl-CoA:cholesterol acyltransferase activity and reduction of serum

- LDL/VLDL cholesterol levels by glucobrassicin derivatives. *Biochemical Pharmacology*, 47, 359–364.
- Folch J., Lees M., Sloane G.S.H. (1975): A simple method of the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, 226, 497–509.
- Foster L.B., Dunn R.T. (1973): Stable reagents for determination of serum triglycerides by colorimetric Hatzsch condensation method. *Clinical Chemistry*, 19, 338–340.
- Gibbons G.F., Islam K., Pease R. (2000): Mobilisation of triacylglycerol stores. *Biochimica et Biophysica Acta*, 1483, 37–57.
- Hugget A.S.G., Nixon D.A. (1957): Use of glucose-oxidase, peroxidase and o-dianisidine in determination of blood and urinary glucose. *Lancet*, 2, 368–370.
- Kim D.J., Han B.S., Ahn B., Hasegawa R., Shirai T., Ito N., Tsuda H. (1997): Enhancement by indole-3-carbinol of liver and thyroid gland neoplastic development in a rat medium-term multiorgan carcinogenesis model. *Carcinogenesis*, 18, 377–381.
- LeBlanc G.A., Stuart J.D., Dunn S.E., Baldwin W.S. (1994): Effect of the plant compound indole-3-carbinol on hepatic cholesterol homeostasis. *Food and Chemical Toxicology*, 32, 633–639.
- Manson M.M., Ball H.W., Barrett M.C., Clark H.L., Judah D.J., Williamson G., Neal G.E. (1997): Mechanism of action of dietary chemoprotective agents in rat liver: induction of phase I and II drug metabolizing enzymes and aflatoxin B1 metabolism. *Carcinogenesis*, 18, 1729–1738.
- Nankee M.N., Verhagen H., van Poppel G., Rompelberg C.J.M., van Bladeren P.J., Miller N.E. (1996): Do dietary phytochemicals with cytochrome P-450 enzyme-inducing activity increase high-density-lipoprotein concentrations in humans? *American Journal of Clinical Nutrition*, 64, 706–711.
- Okulicz M., Bialik I., Chichłowska J. (2005): The time-dependent effect of gluconasturtiin and phenethyl isothiocyanate on metabolic and antioxidative parameters in rats. *Journal of Animal Physiology and Animal Nutrition*, 89, 367–372.
- Park J.Y., Shigenaga M.K., Ames B.N. (1996): Induction of cytochrome P450A1 by 2,3,7,8-tetrachlorodibenzo-p-dioxin or indolo(3,2-b)carbazole is associated with oxidative DNA damage. *Proceedings of the National Academy of Sciences USA*, 93, 2322–2327.
- Richmond W. (1973): Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. *Clinical Chemistry*, 19, 1350–1356.
- Rodbell M. (1964): Metabolism of isolated fat cells. *The Journal of Biological Chemistry*, 239, 375–380.
- Stresser D.M., Williams D.E., Griffin D.A., Bailey G.S. (1995): Mechanisms of tumor modulation by indole-3-carbinol. Disposition and excretion in male Fischer 344 rats. *Drug Metabolism and Disposition*, 23, 965–975.
- Szkudelska K., Nogowski L., Szkudelski T. (2000): Genistein affects lipogenesis and lipolysis in isolated rat adipocytes. *The Journal of Steroid Biochemistry and Molecular Biology*, 75, 265–271.
- Szkudelska K., Szkudelski T., Nogowski L. (2002): Daidzein, coumestrol and zearalenone affect lipogenesis and lipolysis in rat adipocytes. *Phytomedicine*, 9, 338–345.
- Szkudelska K., Nogowski L., Nowicka E., Szkudelski T. (2007): *In vivo* metabolic effects of naringenin in the ethanol consuming rat and the effect of naringenin on adipocytes *in vitro*. *The Journal of Animal Physiology and Animal Nutrition*, 91, 91–99.
- Szkudelski T., Szkudelska K. (2002): Streptozotocin induces lipolysis in rat adipocytes *in vitro*. *Physiological Research*, 51, 255–259.
- Wortelboer H.M., de Kruif C.A., van Iersel A.A.J., Falke H.E., Noordhoek J., Blaauboer B.J. (1992): Acid reaction products of indole-3-carbinol and their effects on cytochrome P450 and phase II enzymes in rat and monkey hepatocytes. *Biochemical Pharmacology*, 43, 1439–1447.

Received: 2008–06–30

Accepted after corrections: 2008–11–11

---

*Corresponding Author*

dr. Monika Okulicz, Department of Animal Physiology and Biochemistry, Faculty of Animal Breeding and Biology, University of Life Sciences in Poznań, Wołyńska 35, 60 637 Poznań, Poland  
Tel. +48 618 487 196, fax +48 618 487 197, e-mail: mokolicz@au.poznan.pl

## INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

Slezská 7, 120 56 Prague 2, Czech Republic

Tel.: + 420 227 010 111, Fax: + 420 227 010 116, E-mail: redakce@uzpi.cz

In this institute scientific journals dealing with the problems of agriculture and related sciences are published on behalf of the Czech Academy of Agricultural Sciences. The periodicals are published in English with abstracts in Czech.

Journal	Number of issues per year	Yearly subscription in USD
Plant, Soil and Environment	12	285
Czech Journal of Animal Science	12	285
Agricultural Economics (Zemědělská ekonomika)	12	285
Journal of Forest Science	12	285
Veterinární medicína (Veterinary Medicine – Czech)	12	285
Czech Journal of Food Sciences	6	150
Plant Protection Science	4	85
Czech Journal of Genetics and Plant Breeding	4	85
Horticultural Science	4	85
Research in Agricultural Engineering	4	85
Soil and Water Research	4	85

**Subscription to these journals be sent to the above-mentioned address.**

## INSTRUCTIONS TO AUTHORS

The journal publishes original scientific papers and critical reviews of articles in English. Manuscripts should have abstracts (including keywords). The author is fully responsible for the originality of the paper, its subject, and its formal correctness. The author's declaration that the paper has not been published anywhere else should be enclosed. The Board of Editors decides on the publication of papers, taking into account peer reviews, scientific importance, and manuscript quality. Good laboratory practices and ethical rules must be followed. The SI international system of measurement units should be used. Manuscripts must be grammatically and linguistically correct, and authors whose native language is not English are advised to seek the help of a native English-speaker. Manuscripts containing language errors are disfavored in the reviewing process and may be returned to the author for rewriting before peer review and/or before acceptance.

Only manuscripts assessed by leading experts in the field will be published. If such reviews are not available within four months after registration of the manuscript, the peer review process is terminated, and the authors are notified. They can resubmit the manuscript, after its thorough revision and/or update, either to the Czech Journal of Animal Science or another journal for a new assessment. This should eliminate a long waiting period and probable rejection of the manuscript.

If a revision of the manuscript following the recommendation of the reviewers is requested, the modified manuscript must be re-submitted within three weeks. The authors may, however, request an extension of the re-submission deadline if necessary. All parts of the manuscript, including tables and figures (even unchanged) must be re-submitted. A detailed reply by the authors to every point of the reviewer's recommendations must be attached to the revision manuscript. It is not necessary to accept all the requests of the reviewers, but a clear explanation of why the reviewers' comments were not accepted must be provided. If the deadline for re-submission is missed, the paper will be removed from the reviewing process.

The proof reading must be returned within two days. Only errors originating during preparation of the document for printing can be corrected. Standard proof marks will be used. No changes in the manuscript after acceptance for publication are permitted.

Manuscripts should be sent by e-mail as attachments. Alternatively, they can be submitted in duplicate in hard copy, and a properly labelled Compact Disk (CD) with identical contents, including figures, should be enclosed.

**Copyright.** The journal is protected by copyright held by the publisher after the manuscript has been accepted for publication. As regards the transfer of rights, the corresponding author assumes responsibility for all the authors. No part of this publication may be reproduced, stored, or transmitted in any form or by any means without the written permission of the publisher.

**Manuscript layout.** The Microsoft (MS) Word for Windows word-processing software should be used for creating the text in non-formatted style strictly following the journal layout. If any abbreviations are used in the paper, they must be appropriately explained when they are used in the text for the first time. It is not advisable to use any abbreviation in the title of the paper or in the abstract. Tables, graphs and other Word documents are to be submitted on separate pages appended to the article. The document must not be formatted in columns, heading styles, etc. This unique MS Word file must be saved under the first author's surname only. In the printed version lines should be numbered. Graphs should be provided in MS Excel, and they should be stored with the original data. Photographs and autotypes should be submitted in high resolution (min. 300 dpi) TIFF or JPG format. All tables, graphs and photos should be numbered in the order in which they are included in the text, using Arabic numerals.

**The Title of the Paper** should be short and informative, and no subtitles or numbering of "serial" articles (Part I, Part II, etc.) should be used.

**The Abstract** should not have more than 500 words. It should contain important information on methods used to solve the problem, a clear description of results and their statistical significance, and brief and unambiguous conclusions drawn from the results. References and discussion of the results should not be included in the abstract.

**Keywords** should not repeat nouns used in the title and should describe the studied problem as best as possible.

**The Introduction** section should provide information on the present state of research in the field concerned and on the objective of the study. It should also include references to literary sources used by the authors to document their present findings, but not all literary sources that have been published to date. References in the text should agree with those in the list of references. It is recommended to include references to papers from peer periodicals only. Citations from non-available sources (reports, national journals, proceedings, thesis, etc.) should be omitted. Papers published by one or two authors are to be cited by their names, those published by three or more authors by the name of the first one, et al. If more than one paper by the same author/two authors/first author, et al., published in the same year is cited, they should be differentiated by YEARa,b,c both in the text and the list of References. Names and year of publication are to be cited by including them in the text directly, e.g. "...as published by Brown (1995)" or indirectly – citing authors and year of publication in parentheses (Green and Grey, 1996), (Jakl et al., 2002). Several papers cited together should be arranged according to the year of publication starting with the oldest one.

**Material and Methods.** All preliminary material, conducted experiments, their extent, conditions and course (experimental design) should be described in detail in this section. All original procedures that were used for the processing of experimental material and all analytical methods used for evaluation should also be detailed. Data verifying the quality of the acquired data should be indicated for the methods used. The entire methodology is to be described only if it is an original one; in other cases it is sufficient to cite the author of the method and to mention any particular differences. Methods of statistical processing including the software used should also be listed in this section.

**Results and Discussion.** The results obtained from the experiments including their statistical evaluation and any commentary should be presented graphically or in tables in this section. The author should confront partial results with data published by other authors, whose names and year of publication are to be cited by including them in the text directly or indirectly.

**References** should be arranged in alphabetical order according to the surname and initials of authors. The year of publication cited in parentheses, the full title of the paper in English with the language of publication in parentheses, e.g. (in Czech) should follow. The title of the periodical should be preferably typed in full. Use of official ISI Journal Citation Reports or Current Contents abbreviations is an alternative but should be used only in exceptional cases.

In the case of books or proceedings, the title should be followed by the name of the publisher, its location (Paris, New York, etc.) and the total number of pages.

Only papers cited in the text should be included in the list of references. All names of the authors must be printed in English transcription without non-English letters. Authors are responsible for the accuracy of their references.

**Examples of references in the list:**

Brown J. (1995): Estradiol determination in post-partum sows. *Journal of Endocrinology*, 198, 155–169.

Gabler M.T., Heinrichs A.J. (2003): Dietary protein to metabolizable energy ratios on feed efficiency and structural growth of prepubertal Holstein heifers. *Journal of Dairy Science*, 86, 268–274.

Papers published in monographs or proceedings should be cited as follows:

Kalab J. (1995): Changes in milk production during the sexual cycle. In: Hekel K. (ed.): *Lactation in Cattle*. Academic Press, London, 876–888.

Janson L., Ahlin K.A. (1992): Postpartum reproductive performance in cattle selected for high and low fat content. In: Proc. 43<sup>rd</sup> Annu. Mtg., European Association for Animal Production (EAAP), Madrid, Spain, 93–95.

**The Corresponding Author** should include his or her full name including all academic, scientific and pedagogic titles and the detailed address of the institution with postal code, telephone and fax numbers, and e-mail address. The author who is responsible for any correspondence with the journal should be clearly indicated.

**The Declaration of the Authors** must be carefully completed and signed by the first author.

**Offprints:** Free reprint in Portable Document Format (PDF) sent via e-mail as an attachment.

**Compliance with these instructions is obligatory for all authors. If a manuscript does not comply exactly with the above requirements, the editorial office will not accept it for consideration and will return it to the authors without reviewing it.**