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Original article

Effect of some phytoestrogens on metabolism of rat adipocytes

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Abstract — The aim of this experiment was to evaluate the direct effect of genistein, daidzein and zearalenone on basal and hormone-induced lipogenesis and lipolysis in isolated rat adipocytes. In lipogenesis, daidzein and zearalenone were used at concentrations of 0.01, 0.1 and 1 mmol·L^{-1} and genistein at concentrations of 0.01, 0.3, 0.6 and 1 mmol·L^{-1}. In lipolysis, concentrations of tested compounds were 0.01, 0.1 and 1 mmol·L^{-1}. All tested compounds clearly inhibited basal and insulin (1 nmol·L^{-1}) stimulated lipogenesis. Basal lipolysis was particularly enhanced by genistein and daidzein at its higher concentrations. The ability of zearalenone to potentiate of basal lipolysis was less marked. Epinephrine (1 µmol·L^{-1})-stimulated lipolysis was inhibited by genistein at 1 mmol·L^{-1}. At a concentration of 0.1 mmol·L^{-1} daidzein also augmented epinephrine-stimulated lipolysis and at its highest concentration exhibited an inhibitory effect, similar to genistein. Zearalenone reduced stimulated lipolysis, particularly at the highest concentration. © Inra/Elsevier, Paris.

phytoestrogen / adipocyte metabolism

Résumé — Effet de quelques phytoestrogènes sur le métabolisme des adipocytes du rat. Le but de ce travail était d'examiner les effets directs de la génistéine, de la daidzéine et de la zéaralénone sur la lipolyse et la lipogenèse basale et après stimulation par l'insuline ou l'adrénaline sur des adipocytes isolés de rat. Dans le cas de la lipogenèse, la daidzéine et la zéaralénone ont été ajoutées aux concentrations de 0,01, 0,1 et 1 mmol·L^{-1} et la génistéine aux concentrations de 0,01, 0,3, 0,6 et 1 mmol·L^{-1}. Dans le cas de la lipogenèse, les concentrations testées étaient les mêmes pour chaque composé : 0,01, 0,1 et 1 mmol·L^{-1}. Tous les composés examinés réduisaient nettement la lipogenèse basale ou après stimulation par l'insuline (1 nmol·L^{-1}). La lipolyse basale était particulièrement augmentée par la génistéine et la daidzéine à leur plus forte concentration. L'efficacité de la zéaralénone sur la lipolyse basale était plus faible. La lipolyse après stimulation par l'adrénaline (1 µmol·L^{-1}) a été diminuée par la génistéine à sa plus forte concentration. La daidzéine (0,1 µmol·L^{-1}) augmentait aussi la lipolyse. À sa plus forte concentration, la daidzéine a exercé un effet contraire similaire à celui de la génistéine. La zéaralénone diminuait la lipolyse, en particulier à forte concentration. © Inra/Elsevier, Paris.

phytoestrogène / métabolisme de l'adipocyte

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1. INTRODUCTION

Recently, a great deal of interest has been directed towards the findings that certain food compounds exhibit estrogen-like activity. They are present in plants regularly consumed by animals and man, for instance soy, oat, barley, rye, wheat, corn, alfalfa, clover and others [9]. Because of their origin, they are called phytoestrogens. The principal phytoestrogens are genistein, daidzein, biochanin A, formononetin, coumestrol and mycoestrogen–zearalenone which is produced by Fusaria, a common field organism which also proliferates in poorly stored grains, oil seeds and hay [9]. The exposure to these compounds can adversely affect some physiological functions. It was observed that they are able to cause hyperestrogenic effects including uterine enlargement in immature animals, estrus cycle abnormalities and fertility problems [4]. Phytoestrogens were also found to participate in other events, e.g. cancer development [12], prevention of osteoporosis [2, 3], corticosteroid synthesis disturbances, reduction of cardiovascular diseases [2], and the protection of LDL against oxidative modifications [11, 14]. There are also some reports about their influence on lipid metabolism; however, this effect is not well known.

The purpose of this work was to ascertain the direct effect of some phytoestrogens (genistein, daidzein and zearalenone) on lipid metabolism in isolated rat adipocytes.

2. MATERIALS AND METHODS

2.1. Preparation of adipocytes

Male Wistar rats weighing 160 ± 5 g and kept in standard conditions, were decapitated. Epididymal fat tissue was pooled and adipocytes were isolated according to the Rodbell method [10] with minor modifications. The tissue was rinsed with 0.8 % NaCl, cut into pieces and transferred into a plastic flask with Krebs-Ringer buffer pH 7.4, containing 3 mmol·L^{-1} glucose, 3 % bovine serum albumin (fraction V), 10 mmol·L^{-1} HEPES and 2 mg·mL^{-1} collagenase (from Clostridium histolyticum, type II). Incubation was performed for 90 min by shaking at 37 °C. After incubation, the cells were rinsed four times with warm (37 °C) collagenase free Krebs-Ringer buffer and then filtered through nylon mesh. Adipocyte counts were performed using a microscope with a Bürker-Türk counting chamber.

2.2. Lipogenesis in adipocytes

Fat cell suspensions (10^6 cells·mL^{-1}) were incubated in plastic tubes at 37 °C with Krebs-Ringer buffer, pH 7.4, containing 3 % BSA, 10 mmol·L^{-1} HEPES, 0.5 μCi of [U-^{14}C] glucose, 5.56 mmol·L^{-1} unlabelled glucose in the absence or presence of 1 nM insulin. Daidzein and zearalenone were used at concentrations of 0.01, 0.1 and 1 mmol·L^{-1}, and genistein at concentrations of 0.01, 0.3, 0.6 and 1 mmol·L^{-1}. The final volume was adjusted to 1 mL. 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 scintilation cocktail (OptiPhase 'Hi Safe' Wallac) and total lipid radioactivity was determined.

2.3. Lipolysis in adipocytes

Adipocytes (about 10^6 cells·mL^{-1}) were incubated with shaking in plastic tubes at 37 °C for 90 min with the Krebs-Ringer buffer containing 3 mmol·L^{-1} glucose, 10 mmol·L^{-1} HEPES and 3 % BSA in the absence or presence of 1 μmol·L^{-1} epinephrine. Phytoestrogens were used at concentrations of 0.01, 0.1 and 1 mmol·L^{-1}. The final volume was adjusted to 1 mL. The glycerol released from adipocytes, reflecting the intensity of lipolysis, was measured using the Boehringer Mannheim test. Insulin (porcine, monocomponent) was from NOVO, Nordisk, [U-^{14}C] glucose was from New England Nuclear, zearalenone from Kodak and genistein from Fluka. All other reagents were purchased from Sigma. Results were statistically evaluated using one-way analysis of variance.

3. RESULTS

3.1. Effect of phytoestrogens on lipogenesis

Table I shows glucose conversion to lipids in adipocytes exposed to phytoestrogens in the absence and presence of insulin. We observed that genistein clearly inhibited basal lipogenesis in a concentration-dependent manner. A similar inhibition was observed for both daidzein and zearalenone. Insulin-stimulated lipogenesis was also strongly reduced by phytoestrogens at all tested concentrations.

3.2. Effect of phytoestrogens on lipolysis

The phytoestrogens also significantly affected lipolysis. *Table II* shows glycerol release from adipocytes exposed to phytoestrogens in the absence and presence of epinephrine. Genistein showed a dose-dependent enhanced effect on basal lipolysis. Daidzein also stimulated basal lipolysis, but only at its higher concentrations. Zearalenone showed a less marked activation of basal lipolysis. Epinephrine-stimulated lipolysis was also influenced by genistein, but only at lower concentrations; at its highest concentration it inhibited this process. 0.1 mmol·L^{-1} daidzein also increased lipolysis stimulation. At its highest concentration it exhibited an opposite effect, similar to genistein. Mycoestrogen–zearalenone reduced stimulated lipolysis, particularly at its highest concentration.

4. DISCUSSION

The performed experiments demonstrate significant effect of genistein, daidzein and zearalenone on lipogenesis and lipolysis in

Table I. The effect of genistein, daidzein and zearalenone on basal and insulin-induced lipogenesis in rat adipocytes. Values are means for six repetitions ± SEM. Means in each column marked with different letters (a, b, c, d, e) differ significantly ($P \leq 0.05$) for each experimental design. Insulin was used at a concentration of 1 nmol·L^{-1}.

Phytoestrogen concentration (mmol·L^{-1})		Glucose conversion to lipids (nmol/10^{6}cells/90 min)	Phytoestrogen concentration (mmol·L^{-1})		Glucose conversion to lipids (nmol/10^{6}cells/90 min)
Control		580 ± 11^a	insulin		667 ± 12^a
Genistein	0.01	533 ± 9^a	insulin +	0.01	611 ± 10^b
	0.3	349 ± 5^b	genistein	0.3	427 ± 3^c
	0.6	227 ± 5^c		0.6	280 ± 6^d
	1	139 ± 5^d		1	181 ± 5^e
Control		615 ± 17^a	insulin		659 ± 6^a
Daidzein	0.01	603 ± 9^a	insulin +	0.01	611 ± 13^b
	0.1	560 ± 24^b	daidzein	0.1	568 ± 8^c
	1	404 ± 15^c		1	463 ± 10^d
Control		812 ± 21^a	insulin		840 ± 18^a
Zearalenone	0.01	735 ± 13^b	insulin +	0.01	747 ± 33^b
	0.1	712 ± 7^b	zearalenone	0.1	745 ± 19^b
	1	484 ± 5^c		1	557 ± 10^c

Table II. The effect of genistein, daidzein and zearalenone on basal and epinephrine-induced lipolysis in rat adipocytes. Values are means for six repetitions ± SEM. Means in each column marked with different letters (a, b, c, d) differ significantly ($P \leq 0.05$) for each experimental design. Epinephrine was used at a concentration of 1 μmol·L^{-1}.

Phytoestrogen concentration (mmol·L^{-1})		Glycerol release (μmol/10^6 cells/90 min)	Phytoestrogen concentration (mmol·L^{-1})		Glycerol release (μmol/10^6 cells/90 min)
Control		0.37 ± 0.02^a	epinephrine		2.37 ± 0.04^a
Genistein	0.01	0.43 ± 0.01^a	epinephrine + genistein	0.01	2.70 ± 0.07^b
	0.1	0.58 ± 0.01^b		0.1	2.79 ± 0.05^b
	1	1.21 ± 0.08^c		1	1.51 ± 0.07^c
Control		0.27 ± 0.01^a	epinephrine		1.18 ± 0.01^a
Daidzein	0.01	0.27 ± 0.01^a	epinephrine + daidzein	0.01	1.17 ± 0.02^a
	0.1	0.31 ± 0.01^b		0.1	1.22 ± 0.01^b
	1	0.35 ± 0.01^c		1	1.03 ± 0.01^c
Control		0.29 ± 0.01^a	epinephrine		1.32 ± 0.03^a
Zearalenone	0.01	0.26 ± 0.01^a	epinephrine + zearalenone	0.01	1.30 ± 0.01^a
	0.1	0.27 ± 0.02		0.1	1.27 ± 0.02^a
	1	0.34 ± 0.02^b		1	1.13 ± 0.03^b

isolated rat adipocytes. It was observed that all these compounds strongly inhibited lipogenesis and enhanced basal lipolysis. The influence of phytoestrogens on cells is as yet, poorly understood. Genistein action was found as the most characterized phytoestrogen in the literature. In other experiments genistein used in similar concentrations was found to inhibit certain responses to insulin in adipocytes [1]. One of the effects is the reduction of the inhibitory action of insulin on lipolysis [1]. An inhibitory effect on both basal and insulin-stimulated lipogenesis of genistein and other tested compounds was observed in our experiments. This inhibitory effect may be expressed either by the reduction of insulin action or in other ways. It is quite possible that the restriction of lipogenesis is closely related to the inhibitory effect of some phytoestrogens, especially genistein, on glucose metabolism in adipocytes [1]. Genistein also restricts glucose transport to adipocytes via induction of conformational changes of the glucose transporter GLUT4 [13]. Thus, restriction of insulin action and reduction of glucose transport and metabolism seem to be the most probable ways of inhibitory action of tested compounds on lipogenesis in isolated rat adipocytes.

Lipolysis enhanced by genistein, daidzein and zearalenone observed in our experiments may be, particularly in the case of genistein, the result of the inhibition of low K_m cAMP phosphodiesterase activity. This effect may cause fat mobilization as Kuppusamy and Das [6] reported for genistein and a few other flavonoids. It was also observed that phytoestrogens act through estrogen receptors [5] and may exhibit estrogen-like activity. Thus, phytoestrogens may, similarly to estrogens [8], activate a catalytic subunit of adenylate cyclase. This activation causes an increase in the cAMP level in fat cells and promotes lipolysis. The increase of cAMP in adipocytes by two different means, inhibition of its decomposition and stimulation of its synthesis, may

explain the increase in lipolysis caused by the tested compounds. At the highest concentration, genistein, daidzein and zearalenone were able to restrict epinephrine-stimulated lipolysis. This finding confirms other observations showing that phytoestrogens may exhibit opposite effects depending on their concentration [15].

The results obtained in this study clearly indicate that genistein, daidzein and zearalenone significantly affect the metabolism of rat adipocytes in vitro in a dose-dependent manner. Genistein was found to be the most potent compound. We also suggest that phytoestrogen action on adipocytes may be one of the reasons for changes in lipid metabolism in the whole organism as it was previously demonstrated for genistein [7]. The exact effect of tested compounds under in vivo conditions is, however, difficult to specify because of their partial conjugation and metabolism to compounds of which activity is not well characterized.

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