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The influence of genistein on insulin, leptin, thyroid hormones and metabolic parameters in mature rats

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ABSTRACT

Genistein is a phytoestrogen and is found in many plants consumed by humans and animals. This isoflavone was found to exert metabolic effects, especially on lipid and carbohydrate metabolism. The aim of this experiment was to determine whether genistein at a dose of 1 and 5 mg/kg body weight administered intragastrically to male and female adult rats changes insulin, leptin, thyroid hormone, and metabolic parameters. The results suggest that genistein has only a slight influence on metabolism. A substantial reduction of triglyceride stores was observed in the skeletal muscles. This effect was sex-dependent and occurred only in females. Moreover, it was demonstrated that genistein at the higher dose decreased blood insulin and leptin levels.

KEY WORDS: genistein, insulin, leptin, metabolism, rats

INTRODUCTION

Genistein, an isoflavone, is a compound naturally occurring in several plants consumed by humans and animals. The highest concentration of this phytoestrogen is in soyabean and soya products such as soya drinks, tofu, isolated soya protein, soya flour and flakes (see review Szkudelska and Nogowski, 2007). Genistein is also present in beans, peas, clover, lentil, and lucerne (Price and Fenwick, 1985). After consumption, genistein appears in the blood. In humans consuming large

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amounts of soya products, the plasma genistein concentration is in the range of 1-4 μM (Adlercreutz et al., 1993; Xu et al., 2000).

Genistein has an aromatic ring with three hydroxyl groups (other isoflavones such as daidzein, formononetin, and biochanin A have one or two hydroxyl groups). This structure enables it to bind to the estrogen receptor (ER). Genistein has an affinity to both ER subtypes-ERα and ERβ, but its affinity for ERβ is significantly higher (Kuiper et al., 1997, 1998).

This soya-derived isoflavone demonstrates pleiotropic action in the organism-it has both estrogenic and antiestrogenic effects (Price and Fenwick, 1985). Genistein provides many health benefits. Numerous studies indicate that this plant compound exhibits anticancerogenic activity and plays a protective role against breast cancer (it reduces cell proliferation), prostate and colon cancer (Knight and Eden, 1995; Lamartiniere, 2000; Rannikko et al., 2006). Furthermore, genistein possesses antioxidant properties, reduces the risk of cardiovascular disease, osteoporosis and it may alleviate some post-menopausal problems (see review Szkudelska and Nogowski, 2007).

Genistein has been shown to exert an anti-obesity effect. Naaz et al. (2003) demonstrated that this phytoestrogen reduced adipose tissue weight and decreased lipoprotein lipase gene expression in adipose tissue in mice. Soya protein is also able to induce beneficial changes in lipid metabolism. In a study conducted on male rabbits consuming soya isoflavones, plasma total lipids, total cholesterol, triglycerides, LDL- and VLDL-cholesterol were decreased (Yousef et al., 2004). Pure genistein also shows beneficial effects. Nogowski et al. (1998) found that genistein decreased the triglyceride concentration in blood and skeletal muscle in ovariectomized rats fed a diet with genistein for 14 days. In an experiment performed on male rats receiving the tested compound, serum HDL-cholesterol also tended to be increased (Szkudelska et al., 2003). Moreover, this isoflavone has an influence on metabolism of isolated rat adipocytes. It substantially restricted lipogenesis and enhanced epinephrine-induced lipolysis (Szkudelska et al., 2002).

The literature data concerning the effects of genistein on metabolism have not been fully elucidated. Many experiments performed to test the metabolic action of genistein were conducted on ovariectomized rats (Nogowski et al., 1998) or on pregnant animals (Nogowski et al., 2006). Recently, the effects of genistein on carbohydrate and lipid metabolism in immature female rats were also described (Nogowski et al., 2007).

The aim of this experiment was to determine the influence of seven-day genistein administration on insulin, leptin, thyroid hormones, and metabolic parameters in sexually mature female and male rats.

MATERIAL AND METHODS

Animals and measurements

The experiment was performed according to the guidelines accepted by the Local Ethics Committee for Investigations on Animals.

Male and female Wistar rats weighing about 300 g were used in the experiment. The animals were kept under standard conditions, at a constant temperature (21±1°C) with a 12-h dark-light cycle in an air-conditioned room. Rats were fed a soya-free diet (Labofeed B, Poland) *ad libitum*. Males and females were divided into three groups (n=9). Animals in the control group received the vehicle, i.e. a dimethyl sulphoxide (DMSO):water mixture (4:6 v/v), whereas rats in the second and third groups received genistein dissolved in the vehicle in the amount of 1 or 5 mg/kg body weight (BW), respectively. The vehicle and genistein solutions (Sigma) were given intragastrically (0.5 ml/100 g BW) once a day for seven days. The animals were decapitated and their blood serum, livers and muscles were collected and stored (-80°C) until analysis.

Analysis

Serum hormone concentrations were assayed radioimmunologically using kits specific for rat hormones: insulin and leptin from Linco Research Inc. (USA) and thyroid hormones from CIS Bio International (France). Glucose was determined enzymatically using glucose oxidase, peroxidase and o dianisidin. Serum free fatty acids were assayed by the method of Duncombe (1964), serum triglycerides by the method of Foster and Dunn (1973), and serum cholesterol (total, free, and estrified) by the enzymatic method described by Richmond (1973). HDL-cholesterol was assayed in serum after separation of high density lipoproteins using polyethylene glycol. Liver and muscle glycogen was extracted and hydrolysed by amyloglucosidase (Szkudelska et al., 2003). Liver and muscle triglycerides and cholesterol content were determined similarly as in serum after extraction of total lipids using the method of Folch et al. (1975). Solvent was evaporated from obtained extracts before cholesterol was determined.

Statistical analysis

All results obtained in the experiment were evaluated statistically using one-way analysis of variance and Duncan's multiple range test at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS

Genistein given at a dose of 1 or 5 mg/kg BW for seven days had a slight effect on lipid metabolism in male and female adult rats. Serum insulin and leptin were also changed by this compound. Genistein administered at a dose of 5 mg/kg BW significantly decreased the blood insulin concentration (Tables 1 and 3). It was also observed that genistein at the higher dose markedly decreased the blood leptin level. The serum concentrations of total and free thyroxine (T4) and triiodothyronine (T3) were not affected by genistein (Tables 1 and 3).

Table 1. The influence of genistein on serum hormones in mature male rats

Blood serum	Control	Genistein 1 mg/kg BW	Genistein 5 mg/kg BW
Insulin, ng/ml	2.88 ± 0.32^{A}	2.12 ± 0.22	2.06 ± 0.19^{B}
Leptin, ng/ml	4.98 ± 0.25^{A}	4.18 ± 0.54	3.99 ± 0.16^{B}
Triiodothyronine			
total, ng/ml	0.45 ± 0.04	0.39 ± 0.03	0.39 ± 0.02
free, pg/ml	7.15 ± 0.39	6.81 ± 0.39	6.87 ± 0.25
Thyroxine			
total, ng/ml	49.05 ± 2.74	44.08 ± 2.76	49.09 ± 2.50
free, pg/ml	34.04 ± 2.11	32.56 ± 1.99	31.63 ± 1.97

genistein was dissolved in DMSO:water mixture (4:6 v/v; 0.5 ml/100 g BW) and was administered intragastrically for seven days. Animals in control group received vehicle. Values are means ± SEM for 9 rats. Means in the same row with different letter superscripts differ significantly ($P \leq 0.05$)

Table 2. The influence of genistein on metabolic parameters in mature male rats

Blood serum	Control	Genistein 1 mg/kg BW	Genistein 5 mg/kg BW
Glucose, mmol/l	4.35 ± 0.23	4.40 ± 0.15	4.38 ± 0.13
Free fatty acids, mmol/l	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Triglycerides, mmol/l	1.70 ± 0.09	1.54 ± 0.05	1.60 ± 0.08
Cholesterol, mmol/l			
total	1.45 ± 0.14	1.49 ± 0.17	1.56 ± 0.11
free	0.65 ± 0.05	0.68 ± 0.04	0.77 ± 0.04
oesterified	0.80 ± 0.10	0.81 ± 0.15	0.79 ± 0.10
cholesterol HDL	2.70 ± 0.28	3.21 ± 0.31	2.93 ± 0.22
cholesterol HDL/total	1.91 ± 0.17	2.30 ± 0.27	1.94 ± 0.19
Liver			
glycogen, g/kg	37.73 ± 5.44	31.68 ± 4.37	34.43 ± 5.25
cholesterol, mmol/kg	10.77 ± 0.43	10.75 ± 0.56	10.68 ± 0.29
triglycerides, mmol/kg	13.04 ± 0.49	12.15 ± 0.51	11.75 ± 0.47
Muscle			
glycogen, g/kg	1.29 ± 0.19	1.20 ± 0.16	1.26 ± 0.15
cholesterol, mmol/kg	2.42 ± 0.20	2.45 ± 0.19	2.51 ± 0.15
triglycerydes, mmol/kg	41.35 ± 9.18^{A}	28.38 ± 9.47	20.28 ± 3.22^{B}

genistein was dissolved in DMSO: water mixture (4:6 v/v; 0.5 ml/100 g BW) and was administered intragastrically for seven days. Animals in control group received vehicle. Values are means ± SEM for 9 rats. Means in the same row with different letter superscripts differ significantly ($P \leq 0.05$)

Tabele 3. The influence of genistein on serum hormones in mature female rats

Blood serum	Control	Genistein 1 mg/kg BW	Genistein 5 mg/kg BW
Insulin, ng/ml	1.99 ± 0.09^{A}	1.75 ± 0.13	1.18 ± 0.21^{B}
Leptin, ng/ml	3.85 ± 0.18^{A}	3.35 ± 0.26	3.15 ± 0.11^{B}
Triiodothyronine			
total, ng/ml	1.09 ± 0.03	0.99 ± 0.06	1.07 ± 0.06
free, pg/ml	4.90 ± 0.23	5.31 ± 0.21	5.25 ± 0.28
Thyroxine			
total, ng/ml	99.59 ± 8.67	95.85 ± 9.95	87.07 ± 8.63
free, pg/ml	21.07 ± 1.33	21.66 ± 1.11	24.69 ± 1.70

genistein was dissolved in DMSO:water mixture (4:6 v/v; 0.5 ml/100 g BW) and was administered intragastrically for seven days. Animals in control group received vehicle. Values are means ± SEM for 9 rats. Means in the same row with different letter superscripts differ significantly (P≤0.05)

Dietary genistein administered at a dose of 1 and 5 mg/kg BW had no influence on blood glucose, free fatty acids, cholesterol, or triglyceride concentrations. The results obtained in our experiment clearly demonstrated that genistein at the higher dose substantially reduced muscle triglycerides in adult rats (Tables 2 and 4).

Tabele 4. The influence of genistein on metabolic parameters in mature female rats

Blood serum	Control	Genistein 1 mg/kg BW	Genistein 5 mg/kg BW
Glucose, mmol/l	4.95 ± 0.14	5.07 ± 0,22	5.40 ± 0.26
Free fatty acids, mmol/l	0.17 ± 0.02	0.12 ± 0.02	0.12 ± 0.01
Triglycerides, mmol/l	1.03 ± 0.03	1.11 ± 0.07	1.08 ± 0.05
Cholesterol, mmol/l			
total	2.17 ± 0.12	2.11 ± 0.12	2.17 ± 0.16
free	0.97 ± 0.14	0.82 ± 0.09	0.84 ± 0.08
oesterified	1.20 ± 0.14	1.29 ± 0.09	1.33 ± 0.10
cholesterol HDL	1.35 ± 0.05	1.25 ± 0.09	1.28 ± 0.11
cholesterol HDL/total	0.62 ± 0.02	0.59 ± 0.03	0.59 ± 0.02
Liver			
glycogen, g/kg	25.37 ± 1.42	34.08 ± 3.35	33.55 ± 3.56
cholesterol, mmol/kg	11.06 ± 0.50	11.32 ± 0.48	11.19 ± 0.43
triglycerides, mmol/kg	13.55 ± 0.99	16.26 ± 0.83	14.64 ± 0.53
Muscle			
glycogen, g/kg	1.40 ± 0.20	1.35 ± 0.19	1.63 ± 0.14
cholesterol, mmol/kg	1.78 ± 0.18	1.71 ± 0.13	1.77 ± 0.11
triglycerides, mmol/kg	49.10 ± 4.51^{A}	19.11 ± 3.08^{B}	21.48 ± 3.39^{B}

genistein was dissolved in DMSO:water mixture (4:6 v/v; 0.5 ml/100 g BW) and was administered intragastrically for seven days. Animals in control group received vehicle. Values are means ± SEM for 9 rats. Means in the same row with different letter superscripts differ significantly (P≤0.01)

DISCUSSION

Genistein administered at a dose of 5 mg/kg BW for 7 days caused a substantial decrease in the blood leptin concentration. Leptin is a hormone that plays an important role in regulation of food intake, body weight and energy status of the organism (Ahima and Flier, 2000). It was demonstrated that genistein (5 mg/kg BW for seven days) diminished the blood leptin level in immature female rats (Nogowski et al., 2007). Similar results were observed in adult male rats receiving genistein at the same dose (5 mg/kg BW) but only for 3 days (Szkudelska et al., 2003). Also, blood leptin levels were reduced in adult male rats consuming a diet enriched in isoflavones (Lephart et al., 2004).

The reason for the decreased level of leptin after genistein intake may be the simultaneous reduction in blood insulin. It is well established that insulin is one of the stimulators of leptin secretion in rat adipocytes (Mueller et al., 1998; Szkudelski et al., 2005). The results obtained in our study clearly indicate that genistein at the higher dose essentially decreased the blood insulin level in female and male rats. There are many results that support such an activity of the investigated phytoestrogen. Szkudelska et al. (2003) demonstrated that genistein may effectively modulate insulin concentrations. This phytoestrogen at a dose of 5 mg/kg BW administered for 3 days diminished blood insulin levels in adult male rats (Szkudelska et al., 2003). A similar effect was observed in immature female rats after 7 days of treatment with genistein (Nogowski et al., 2007).

The decreased blood leptin level observed in the presented experiment may also result from the direct influence of genistein on fat tissue. It is well documented that this isoflavone decreased adipose tissue weight in mature male mice (Penza et al., 2006) and in male rats (Lephart et al., 2004). Genistein also attenuates leptin release from isolated adipocytes (Szkudelski et al., 2005). Moreover, it was proved that the tested compound restricts insulin-stimulated glucose transport in isolated fat cells in rats by causing conformational changes in GLUT4 (Smith et al., 1993). Furthermore, the hypoleptinaemic effect of genistein may be due to attenuation of insulin action in adipocytes. Genistein restricts glucose oxidation in fat cells, thus reducing the amount of ATP, one of the main stimulators of leptin secretion (Abler et al., 1992).

Triiodothyronine and thyroxine are essential hormones in the regulation of the energy balance of an organism, so we investigated the influence of genistein on these thyroid hormones. The tested compound did not alter blood concentrations of total and free thyroid hormones. Several studies provide similar results. This phytoestrogen had no effect on TSH, T3, or T4 levels in rats receiving genistein in a diet for 20 weeks (Chang and Doerge, 2000).

Genistein has been shown to be a compound influencing lipid metabolism in humans and animals (Ae Park et al., 2006). However, the literature data concerning

this subject are inconsistent. Our experiment showed that the effects of genistein on lipid and carbohydrate metabolism are insignificant. Cholesterol, free fatty acids, and glycogen were unaffected by the tested phytoestrogen. Genistein did not change either blood or liver triglyceride concentrations. Some literature data also indicate that genistein has no effects on these parameters. In an experiment conducted on mature male rats receiving genistein at 5 mg/kg BW for three days, Szkudelska et al. (2003) observed that cholesterol, free fatty acids and glycogen contents were not influenced by the soya isoflavone.

Nonetheless, we found that genistein causes a substantial reduction of triglyceride concentrations in skeletal muscle of both male and female rats. A similar effect of genistein was also reported in ovariectomized rats (Nogowski et al., 1998), in adult male rats (Szkudelska et al., 2003), and in immature female rats treated with genistein (Nogowski et al., 2007). The mechanism through which genistein affects triglyceride concentrations in muscle is unknown. One of the reasons may be its stimulatory effect on lipolysis and inhibitory effect on lipogenesis, similarly as in adipose tissue (Szkudelska et al., 2002).

Our experiment was performed on mature male and female rats and it was found that there are differences in the influence of genistein on triglyceride stores between the sexes. In females, the amount of muscle triglycerides was higher than in males. Moreover, the influence of genistein on triglyceride reserves was stronger in female rats. These findings allow the supposition that muscle tissue in females is much more sensitive to genistein than male tissue. The differences between sexes in triglyceride concentrations and amounts after genistein treatment may be due to a different sensitivity to oestrogens.

Szkudelska et al. (2003) demonstrated that oestradiol administered to male rats decreased the triglyceride content in muscle. This effect could be the result of diminished activity of muscle lipoprotein lipase, which is the main enzyme involved in triglyceride metabolism (Ramirez, 1981). Genistein may exert a similar effect on muscle triglycerides as oestradiol, therefore, the genistein-induced reduction in triglycerides may be a consequence of the estrogenic activity of this compound. The observed differences between sexes could also be a consequence, as mentioned above, of diminution of lipoprotein lipase activity (Ramirez, 1981). Estrogens (and genistein) decrease the activity of this enzyme and thus strongly restrict triglyceride contents in female.

CONCLUSIONS

The results obtained in this experiment prove that 7-day-long intragastric administration of genistein may change insulin, leptin, and thyroid hormone levels and parameters of lipid metabolism in both mature females and males.

This compound essentially decreased blood insulin and leptin concentrations and reduced the triglyceride content in skeletal muscles. This latter effect was sex-dependent and occurred only in females. Mechanisms responsible for the detected activity of genistein require further investigations.

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