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Effect of Calorie Restriction Supplemented with Genistein on Serum Levels of Glucose, Lipid Profile and Inflammatory Markers (Resistin and hsCRP) in Obese Rats

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ABSTRACT

It seems that soy isoflavones can improve obesity and reverse subsequent metabolic disorders. In this study we assessed the effect of restriction of calorie supplemented with genistein on diet-induced obese rats. Thirty male Wistar obese rats were divided randomly into 3 experimental groups (n = 10) as follows: group 1: low calorie diet supplemented with 50 mg kg^{-1} genistein, group 2: low calorie diet supplemented with dimethyl sulphoxide (as vehicle) and group 3: obese control rats with *ad libitum* access to standard food. The animals were carefully monitored and weighed daily. After 4 weeks, fasting blood samples were collected and analyzed for biochemical analysis. The results showed that the restriction of calorie intake caused to weight loss and subsequently led to a significant decrease in serum glucose ($p<0.001$), lipid profile ($p<0.001$) and hsCRP levels ($p<0.001$) but had no effect on resistin levels. Although, low calorie diet supplemented with genistein improved the lipid lowering effect of calorie restriction but this had no more effect on glucose, resistin and hsCRP levels. These results strongly suggest that low calorie diet supplemented with genistein is effective in weight management and has hypoglycemic, hypolipidemic and anti-inflammatory activities. However, it is not recommended before further investigations in animals and humans.

Key words: Genistein, obesity, inflammatory markers, lipid profile

INTRODUCTION

The prevalence of obesity worldwide has increased dramatically in the past decade (Lazar, 2005). Obesity is a risk factor for different health problems related to coronary heart disease, diabetes, hyperlipidemia and cancer; and its incidence is rapidly increasing (Zimmermann-Belsing and Feldt-Rasmussen, 2004; Ogden *et al*., 2006). Therefore, there is increased need to develop policies that will be effective for both the treatment and prevention of obesity.

Fat mass can be regulated by different factors, including estrogens which maintain, promote and control the distribution of fat and affect adipose tissue metabolism. The factors that regulate adipose tissue metabolism are of special interest because of rising in obesity and obesity-related problems (Kopelman, 2000). Increasing body of studies has focused on the role of nutritional factors in the etiology, prevention and treatment of obesity (Astrup *et al*., 2008). Among these, studies on

the role of phytoestrogens in obesity have been focused as an important research area in the last 7 years (Cope *et al*., 2008; Orgaard and Jensen, 2008; Rayalam *et al*., 2008).

Phytoestrogens are plant-derived estrogens that can bind to both estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) and mimic the actions of estrogens on target tissues (Naaz *et al*., 2008). The isoflavone genistein (4,5,7 trihydroxyisoflavone) is a phytoestrogen that is found in high concentrations in soy and soy products (Reinli and Block, 1996). It has attracted much attention among medical and public communities because of its possible role in prevention and treatment of many diseases including diabetes, menopausal symptoms, osteoporosis, arthritis, cardiovascular diseases, renal diseases and various types of cancers (Mohammad Shahi *et al*., 2011). Beneficial effects of genistein on glucose and lipid metabolism have also been reported by some studies and are attributed to its effect on Peroxisome Proliferator-Activated Receptor (PPAR) (Mezei *et al*., 2003; Kim *et al*., 2004).

Genistein and other soy isoflavones like daidzein are consumed by Oriental populations at concentrations up to 1 mg kg^{-1} per day and infants fed the soy formula consume even higher quantities of isoflavones (Setchell *et al*., 1997).

Although, several studies have shown that genistein is beneficial for hyperlipidemia and cardiovascular diseases and may help to improve obesity and diabetes (Banz *et al*., 2004; Jones *et al*., 2005) but the results remain controversial and need further investigation. The present study examined the effect of low calorie diet supplemented with genistein on body weight, serum glucose, lipid profile and inflammatory biomarkers including resistin and hsCRP (high sensitive C reactive protein). In this study, we hypothesized that the supplementation of low calorie diet with genistein can synergistically improve the serum glucose and lipid profiles as well as the increased concentrations of resistin and hsCRP in obese rats.

MATERIALS AND METHODS

Animals: Thirty six male Wistar rats (150-170 g), aged 7-8 weeks, were obtained from Physiology Research Center of Jundishapur University of Medical Sciences. The animals were housed under standard conditions (23±4°C, 57±4% humidity and a 12-h light/dark cycle) and had free access to *ad libitum* standard laboratory diet and water for 2 weeks for acclimatization. The study was conducted in consistent with ethical procedures and policies approved by the Animal Care and Use Committee of Jundishapur University of Medical Sciences, Ahvaz, Iran.

High fat diet- induced obesity: Obesity was induced in rats by feeding the rats with a High Fat Diet (HFD) for 6 weeks (Guo *et al*., 2009). The high fat diet consisted of 40% of calorie from fat, 20% from protein and 40% from carbohydrate (Table 1). Each gram of this diet contained 19.6 KJ (4.7 kcal). Body weight and weight of the ingested food were measured once within two days.

After feeding the high-fat diet for 6 weeks, a significant weight gain in HFD group was observed as compared to control group (254±22 g vs. 196±7 g; $p = 0.000$).

Study design: Thirty high-fat induced obese rats were divided randomly into three groups ($n = 10$) and treated as follows: Group 1: obese rats taking low calorie diet supplemented with 50 mg kg^{-1} genistein; Group 2: obese rats taking low calorie diet supplemented with dimethyl sulphoxide (DMSO) and Group 3: obese rats having free-access to standard laboratory diet (as control). Genistein (GE) and DMSO (DM) were given by oral gavage and the treatment continued for four weeks. Food intake and body weight were controlled daily during the intervention. During

Table 1: Composition of the high-fat diet fed to rats

Ingredients	g kg^{-1} diet
Casein (Merck, Germany)	200
D-L methionine (Merck, Germany)	3
Corn starch (Alborz, Iran)	111
Sucrose	370
Wheat bran	50
Corn oil	30
Animal butter	170
Mineral mixture[1] (MP Biomedicals, USA)	40
Vitamin mixture[2] (MP Biomedicals, USA)	12
Cholin bitartrate	2
Fat%	40
Total energy, kJ kg^{-1} diet	19315

[1]Mineral mixture for AIN-76A rodent diet; [2]Vitamin mixture for AIN-76A rodent diet

calorie restriction, the food intake of GE and DM groups were restricted to 40% of the energy available to their respective controls (22.63±5.00 and 23.45±6.01 kcal day^{-1}, respectively, vs. 54.36±7.05 kcal day^{-1}).

Biochemical analysis: At the end of the study, fasting blood samples were collected directly through the heart under light ether anesthesia. Sera were obtained by centrifuging the blood samples at 4000 rpm for 10 min at 4°C and stored in-70°C until assayed. Serum hsCRP and resistin concentrations were assayed by ELISA technique using commercial kits (BioVendor, Czech Republic) according to manufacturer's instructions. Fasting blood glucose, triglycerides (TG), Total Cholesterol (TC) and HDL-C levels were measured enzymatically using standard kits by autoanalyser SA1000. LDL-C level was calculated by Friedwald formula as follows:

LDL cholesterol = Total cholesterol-HDL cholesterol-(triglyceride/5)

Statistical analysis: Statistical analyses were carried out by SPSS 17 program for windows. Data were expressed as Mean±SD. Statistical analysis was performed by one way ANOVA, Independent sample t-test and Paired sample t-test with 95% Confidence Interval (CI). Differences were considered to be statistically significant at $p<0.05$.

RESULTS

The initial and final body weights of experimental groups are summarized in Table 2. As it is shown in the table, the initial body weight of 3 intervention groups were equal and the difference between means was not statistically significant ($p = 0.985$). Restriction of the calorie resulted in a significant weight loss in GE and DM group compared to control group ($p = 0.000$).

The body weight changes during the intervention (4 weeks) in experimental groups are also presented in Fig. 1. At the end of the study, the weight of rats in GE group was lower than that of DM group but it is not statistically significant ($p = 0.628$).

The means of serum glucose and lipid profile are shown in Table 3. As it is indicated in the table, serum glucose levels of obese control rats is significantly higher than those of GE and DM groups ($p = 0.001$ and $p = 0.002$, respectively). But the difference between means of glucose concentration in GE and DM group was not statistically significant ($p = 0.927$).

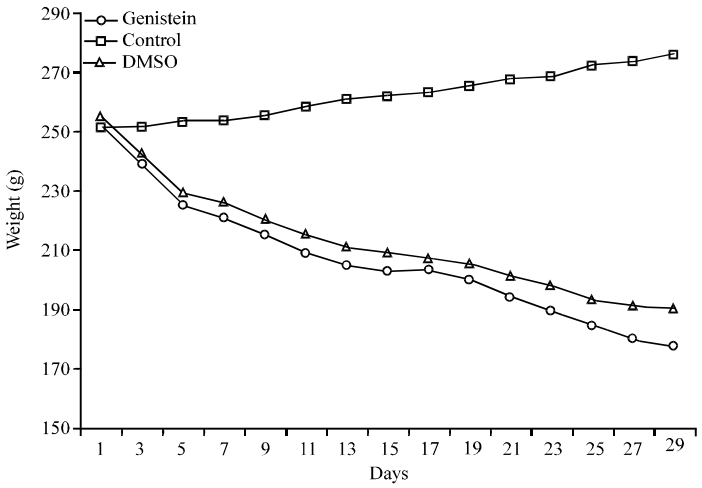

Fig. 1: Effect of calorie restriction supplemented with genistein on body weight changes

Table 2: Means of body weight in experimental groups at the beginning and the end of study

Groups	Initial body weight (g)	Final body weight (g)	P_a	P_b
Control obese rats	251±34	276±38	-	0.000
Calorie restriction+GE[1] (50 mg kg^{-1})	254±16	177±15	0.000	-
Calorie restriction+DM[2] (50 mg kg^{-1})	255±20	190±18	0.000	0.628

All values are expressed as Mean±SD (n = 10). P_a indicates p value vs. control group and P_b indicates p value vs. GE group (independent sample t-test, CI 95%). [1] Genisterin, [2]Dimethyl sulphoxide

Table 3: Means of serum glucose and lipid profile levels in experimental groups at the end of study

Groups	FBS (mg dL^{-1})	TG (mg mL^{-1})	TC (mg mL^{-1})	LDL-C (mg dL^{-1})	HDL-C (mg dL^{-1})
Control obese rats	168.50±13.69**	128.13±14.01**	151.25±12.7**	105.38±14.87**	20.25±2.12**
Calorie restriction+GE[1](50 mg kg^{-1})	137.63±15.11††	61.75±6.86††	101.88±4.15††	57.03±3.78††	32.50±2.33††
Calorie restriction+DM[2](50 mg kg^{-1})	141.70±13.90†	81.00±11.11*††	121.40±10.53*††	78.00±13.21*††	27.20±3.25*††

All values are expressed as Mean±SD (n = 10). Independent-sample t-test was used for statistical significance assessment. †indicates p<0.05 and ††indicates p<0.001 vs. control group; *indicates p<0.05 and **indicates p<0.001 vs. GE group. [1]Genisterin, [2]Dimethyl sulphoxide

Table 4: Means of serum resistin and hsCRP levels in experimental groups at the end of study

Groups	Resistin (ng dL^{-1})	hsCRP (ng dL^{-1})
Control obese rats	1.17±0.33	122±13
Calorie restriction+GE[1](50 mg kg^{-1})	0.98±0.27	82±12*
Calorie restriction+DM[2](50 mg kg^{-1})	1.01±0.33	93±14*

All values are expressed as Mean±SD (n = 10). Independent-sample t-test was used for statistical significance assessment. *indicates p<0.001 vs. control group. [1]Genisterin, [2]Dimethyl sulphoxide

Serum TG, TC and LDL-C were significantly higher in the obese control rats than those of GE and DM groups (p = 0.000 for all). Obese control rats had also significant lower levels of HDL-C than those of treated groups (p = 0.000). Following treatment of obese rats with genistein, the serum levels of TG, TC and LDL-C were decreased and HDL-C concentration was increased significantly (p<0.05).

In the Table 4, the effects of low calorie diet supplemented with genistein on serum levels of resistin and hsCRP are shown. As it is obvious in the table, there was no significant difference in

resistin levels between control group and treated groups (GM and DM groups) ($p = 0.519$ and $p = 0.654$, respectively). However, low calorie diet supplemented with or without genistein significantly decreased the levels of hs-CRP compared to control group ($p<0.001$). We also found that genistein supplementation is not more effective on resistin and hsCRP levels rather than calorie restriction alone.

The present data also indicated a strong relation between hsCRP levels and body weight ($r = 0.653$, $p = 0.000$) but the relation between resistin levels and body weight was not statistically significant ($p>0.05$).

DISCUSSION

Increasing body of evidence suggest that soy and its isoflavones constituents can lead to the reduction in food intake, body weight and adipogenesis (Orgaard and Jensen, 2008; Kishida *et al.*, 2008). However, in our study we found that the supplementation with 50 mg kg^{-1} genistein had no more effect on body weight rather than calorie restriction alone. The results regarding the effect of genistein on body weight and adipogenesis are controversial and need further investigation. Some studies showed that genistein like esterogen inhibit Lipoprotein Lipase (LPL) in adipose tissue (Naaz *et al.*, 2008). Moreover, several evidences have established that isoflavones not only act via ERs but also exert their effects through other pathways, including those regulated by PPARs. Unlike the highly specific ERs, PPARs can bind to various number of ligands and directly affect lipid metabolism by increasing transcription of PPAR-regulated genes (Kishida *et al.*, 2008). Genistein can also act as a tyrosine kinase inhibitor and therefore, contributes to adipocyte differentiation (Kishida *et al.*, 2008). In our study, the effect of calorie restriction on the rate of weight loss was so strong that may be masked the effect of genistein on body weight. Also, it seems that the duration of the intervention and the dose of genistein are critical factors in this context.

In this study we also found that genistein can significantly decrease the levels of TG, TC, LDL-C and increase HDL-C. Kirk *et al.* (1998) demonstrated that the ability of the isoflavones genistein and daidzein to decrease serum cholesterol levels may be attributed to an increase in LDL-C receptor activity which is consistent with other results from a *in vitro* studies (Owen *et al.*, 2004; Mullen *et al.*, 2004).

In the present study we also found that low calorie diet treated groups (GE and DM groups) had lower serum glucose levels in comparison to control group that had *ad libitum* access to food. It seems that restriction of food intake and related decrease in body weight is responsible for low levels of glucose in low calorie diet treated rats versus obese control rats. But, genistein supplementation had no significant effect on serum glucose levels in GE treated group in comparison to DM group. Our finding is in concert with Amani *et al.* (2005). They found that soy isoflavones can ameliorate lipid profile but have no effect on blood glucose levels in hypercholestrolemic rabbits. But, Cederroth *et al.* (2008) showed that treatment of male CD-1 mice with soy phytoesterogens improves insulin sensitivity, at least in part, by activating Adenosine Mono Phosphate Kinase (AMPK) in various tissues, including skeletal muscles and white adipose tissue. Another mechanisem by which genistein can improve glucose levels was defined by Lee (2006). They showed that soy protein and genistein can increase glucokinase and decrease glucose-6-phosphatase in streptozocin-induced diabetic rats (Lee,2006). However, it seems that in our study the rigid restriction of calorie had so strong effect on serum glucose levels that masked the effect of genistein on glucose. Additionally in the Cederroth *et al.*

(2008) study, mice were fed with high phytoestrogen diet from conception and maybe short duration of intervention in our study resulted in null effect of genistein on blood glucose.

In the present study, low calorie diet supplemented with genistein had no effect on serum resistin levels in obese rats. There are a few studies about the effects of isoflavones on resistin levels. Chen *et al.* (2006) investigated the effect of soy isoflavones on gene expression of resistin in insulin resistant rats fed a high-fat diet. They found that treatment with 450 mg kg^{-1} isoflavones can decrease resistin levels. One possible reason that genistein has no effect on resistin levels in our study is that the dose of genistein in our study is lower than Chen *et al.* (2006) study. Another possible explanation is that the duration of our treatment with genistein is short (only 4 weeks) and maybe longer treatment could affect on resistin levels. However, the exact mechanism by which isoflavones decrease resistin levels is not yet determined and further investigations is needed to clarify this.

The results of the present study exhibit lower levels of hsCRP in obese rats taking low calorie diet than those in obese control rats. There has been also a positive correlation between serum hsCRP concentration and glucose levels and body weight in our study which could simply explain that this reduction could be due to the weight loss of low calorie diet- treated groups. This finding is in agreement with other studies. Selvin *et al.* (2007) in a Meta analysis study also reported that weight loss results in reduction of serum hsCRP levels.

In present study we found that genistein supplementation is not more effective on hsCRP levels rather than calorie restriction alone. This result is consistent with other studies (Yildiz *et al.*, 2005; Jenkins *et al.*, 2002). One possible explanation is that hsCRP level is closely related to energy intake and rigid restriction of calorie in our study may be inhibit the effect of genistein on hsCRP level but it seems that this area of research requires further investigations.

CONCLUSION

This study concludes that the restriction of calorie intake is effective in weight management and controlling of glucose and lipid profiles in obese rats and possesses anti-inflammatory effect. Although, low calorie diet supplemented with genistein improves the lipid lowering effect of calorie restriction but this has no more effect on glucose, resistin and hsCRP levels. In this study we used only one dose of genistein, so maybe higher doses of it have favorable effect on these parameters. Soy isoflavones, thus, should be considered as an excellent candidate for future investigation in obesity.

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