Egypt. Acad. J. Biolog. Sci., 5(1): 59 -71 (2013) **B. Zoology**
Email: egyptianacademic@yahoo.com **ISSN: 2090 - 0759**
Received: 15 / 11 /2013 www.eajbs.eg.net

Soy isoflavones reduce adiposity via increasing estrogen receptor beta expression in ovariectomized female rats.

Heba M.A. AbdelRazek[1]; Hend M. Tag[2]; Hekmat M. Tantawy[2] and Hanan Thabet[2]

1- Department of physiology, Faculty Veterinary Medicine, Suez Canal University.
2- Department of Zoology, Faculty of Sciences, Suez Canal University.

ABSTRACT

Soy phytoestrogens have estrogenic activity and are used as a natural substitute for estrogen as a replacement therapy in case of estrogen deficiency. They have many useful activities in vitro and in vivo. However, Evidence is emerging that dietary phytoestrogens play a beneficial role in obesity and metabolic syndrome. The objectives of this study was to determine the effect of soy phytoestrogens on some metabolic parameters including energetic status (weekly food intake and body weight gain), abdominal and brown fat masses, adipocyte size, estradiol receptor beta (ERβ) expression in adipocytes, liver fatty changes, plasma high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides (TG), total cholesterol (TC) and oral glucose tolerance test (OGTT), insulin tolerance test (ITT), plasma leptin and adiponectin levels. A total of 30 ovariectomized female Albino rats were divided into two groups (15 females / group). Control group (C) received phytoestrogen-free casein-based diet and high soy phytoestrogens (HF) group received high phytoestrogens diet containing (27% soybeans) for 7 weeks. The results revealed that high phytoestrogens in diet decreased food intake and body weight gain significantly ($P<0.05$) than control group starting from 4th week and 5th week, respectively. Abdominal fat mass, brown fat masses and adipocytes size were significantly ($P<0.05$) lower in HF group than control. Adipocytes ERβ expression of in HF group was significantly ($P<0.05$) higher than control. The histopathological studies showed fatty infiltration in control group. The expression values of ERβ in adipocytes was significantly higher ($P<0.05$) in HF group than control. Levels of HDL was significantly ($P<0.05$) increased in HF group while LDL, TC and TG were significantly ($p<0.05$) decreased than control. Oral glucose tolerance showed non significant change while insulin sensitivity was significantly improved in HF group. Plasma leptin levels were significantly ($P<0.05$) decreased, while adiponectin levels were increase in HF group than control group. These findings show the high dietary phytoestrogens interfere with adiposity and metabolic syndrome via increasing adipose ERβ expression with consequent reduction in leptin production and increase in adiponectin level that improves insulin sensitivity in ovariectomized female rats.

Keywords: Phytoestrogens , lipid profile, ERβ, leptin, adiponectin, overiectomized rats.

INTRODUCTION

Recently, adipose tissue was shown to be a major endocrine system that produce soluble mediators (adipokines) that play a role in energy homeostasis, lipid metabolism, immune response, and reproduction (Badman & Flier 2005 and Kershaw & Flier 2004 and Tolba, 2013). From these adipokines, leptin which plays a key role in regulating energy intake and expenditure, including appetite and hunger,

metabolism, and behavior. It is one of the most important adipose-derived hormones (Brennan and Mantzoros 2006) it acts on the brain and peripheral organs to regulate energy homeostasis and the neuroendocrine axis (Ahima and Osei, 2008). Adiponectin, Another adipokine, which has been postulated to be an important mediator of the interaction between adiposity and insulin sensitivity (Cnop *et al*., 2003) through regulation of glucose and lipid metabolism by targeting the liver and skeletal muscle. (Ahima and Osei, 2008). This hormone plays a role in the suppression of the metabolic derangements that may result in type 2 diabetes (Ukkola and Santaniemi 2002), obesity and atherosclerosis, (Díez and Iglesias 2003), non-alcoholic fatty liver disease (NAFLD) and an independent risk factor for metabolic syndrome (Renaldi *et al*., 2009).

Estrogens promote, maintain, and control the typical distribution of body fat and adipose tissue metabolism, through a still unknown mechanism. These steroids are known to regulate fat mass by increasing lipolysis through the modulation of the expression of genes that regulate adipose deposition (lipogenesis) and differentiation and adipocyte metabolism (Cooke *et al.,* 2001; Cooke & Naaz, 2004). This regulatory mechanism occurs mainly through estrogen receptors (ERα and ERβ), which also mediate the action of several nutritional compounds such as phytoestrogens.

Phytoestrogens are bioactive molecules present as nutritional constituents of commonly consumed vegetables. Their name derives from the fact that they can bind to estrogen receptors and induce an estrogenic/antiestrogenic response in target tissues (Kuiper *et al*., 1998). The isoflavones genistein and daidzein are among the most abundant phytoestrogens in human and animal diets and are found predominantly in legumes like soy. Goodman-Gruen and Kritz-Silverstein (2001) revealed that the consumption of isoflavones – genistein and daidzein were known by their ability to reduce body mass indexes, fasting insulin concentration, increased HDL cholesterol (Nogowski, *et al.,* 1998; Potter *et al*., 1998 and Sanders *et al.,* 2002) lower total cholesterol, LDL cholesterol (Potter *et al*., 1998; Merz-Demlow *et al.,* 2000; Teede *et al.,* 2001; Wangen *et al*., 2001; Jayagopal *et al*., 2002; Lemay *et al*., 2002).

The estrogenic activity of phytoestrogens was depending on its concentration (Wilson *et al*., 2004), endogenous estrogen levels (Ratna 2002), and gender (Faughnan *et al*., 2004). *In vitro* studies showed that, at low doses, genistein efficiently binds both estrogen receptors, with high affinity to ERβ (Kuiper *et al*., 1998). However, high doses genistein was reported to act as a tyrosine kinase inhibitor (Huang *et al*., 1992 ; Hong *et al*., 2005), an antioxidant (Hwang *et al*., 2003), and a steroid-metabolizing enzyme modulator (Atkinson *et al*., 2003). In addition, it may inhibit the action of estrogen receptors by acting through nuclear receptors such as the peroxisome proliferator-activated receptors (PPARs) (Dang and Lowik 2004). Furthermore, recent studies on adipose tissue in women (Goodman-Gruen, 2003; Kritz-Silverstein 2003) and female mice (Naaz *et al*., 2003) indicate that genistein inhibits adipose deposition and decreases adipose mass, through regulation of the expression of specific genes (Penza *et al*, 2006). Genistein and daidzein were also found to inhibit lipogenesis and stimulate lipolysis in rat adipocytes. The previous studies on the influence of genistein and daidzein on metabolism of fat cells suggests the possibility of its effect on leptin and adiponectin secretion (Yanagisawa *et al*., 2012). Accordingly the aim of this study was to put an insight view on the effect of dietary phytoestrogens in estrogen deprived status on energy balance through determination of food intake, weight gain, lipid profile, abdominal fat mass %, brown fat mass% and adipocytes size. In addition to their relation to expression of ERβ in adipocytes, oral glucose tolerance test (OGTT),

insulin levels and insulin tolerance test (ITT) and plasma leptin and adiponectin hormone levels as factor regulating the energy status at high doses.

MATERIALS AND METHODS

Animal care:

Thirty female Albino rats aged 13 weeks old and mean weight 130.9±10.8 g were used in this study and housed in cages (4 females in each) under standard laboratory conditions. They were kept at room temperature (28±2°C) under natural day light rhythm two weeks prior surgical interference to ovariectomy. The animals were accessed to casein based diet and tap water freely. They received humane care and experiments were carried out according to the criteria outlined by Faculty of Veterinary Medicine, Suez Canal University.

Ovariectomy

Thirty female Albino rats (5 month) weighing approximately 180.9±11.4 g were anathaesized by an intraperitoneal injection of thiopental sodium 50 mg/ kg. Ovariectomy was preceded by a midline dorsal skin incision, 3 cm long, approximately half way between the middle of the back and the base of the tail after placing the animal on its ventral surface. Incision of the muscles was made at linea Alba. The ovary was found, surrounded by a variable amount of fat after accessing to peritoneal cavity. The blood vessels were ligated at the connection between the Fallopian tube and the uterine horn was cut and the ovary moved out. Suturing to muscle layer then to skin was performed by simple continuous suture using vicryl 4/0 (Lasota and Danowska-Klonowska 2004). Animals were given broad spectrum antibiotic (amoxicillin, 10 mg/kg) for 3 successive days after ovariectomy and continued on casein based diet.

Experimental groups

After 3 weeks from ovariectomy, the ovariectomized female rats were divided randomly into two groups: Control group (C), n= 15, they were fed on casein based diet and high phytoestrogens group (HF), received high phytoestrogens diet. All diets were formulated to fulfill all the nutritional requirements of adult rat (Table 1) according to NRC (1995) and were offered for 49 days. Weekly food intake and weekly body weight gain were recorded.

Table 1: Diet composition

INGREDIENTS	CONTROL %	HIGH PYTOESTROGEN %
Yellow corn	40.59	35.04
Corn gluten	15.00	-
Soybean*	-	26.41
Casein	5.00	5.00
Sucrose	22.43	22.32
Starch	7.63	4.16
Cellulose	1.30	0.17
Corn oil	5.00	-
Soybean oil	-	5.00
Ground limestone	1.02	1.04
Dicalcium phosphate	0.34	-
Common salt	0.13	0.13
Premix	0.30	0.30
Methionine	0.30	0.43
Lysine	0.26	-
Tryptophan	0.70	-
Total	100.00	100.00

*Soybean was autoclaved at 110^0c for 30 minutes according to (Westfall and Hauge, 1948) to inactivate trypsin inhibitor, tannins, saponins, phytate, protease inhibitors, lectins and goitrogens.

Blood and tissue sampling

At the end of experiment, the rats were fasted overnight and weighed then sacrificed under effect of light anaethesia for obtaining blood. Whole blood was collected on EDTA tubes then centrifuged at 3000 *g* for 20 min for obtaining plasma then stored at -20^{o}c for determination of lipid profile, leptin and adiponectin levels. Visceral fat was collected from the superficial area covering the alimentary tract and the uterus, was removed and weighed, immediately after blood sample collection. Brown fat also was dissected and weighed. Samples from liver and abdominal fat will kept in 10% formalin saline for histopathology and immunohistochemistry.

Lipid profile

Plasma levels of high-density lipoprotein cholesterol (HDL), total cholesterol (TC) and triglycerides (TG) were measured using enzymatic calorimetric kits (Cat. No. 0599, Stanbio Laboratory, USA, Cat. No. 304710050, ELITech Diagnostic, France and and (Cat. No. 303113050, ELITech Diagnostic, France), respectively according to (Treitz, 1990). Plasm low density lipoprotein cholesterol LDL-C was calculated by Friedwald formula described by Davidson *et al.*, 2009.

LDL-C = Total Cholesterol- (Triglycerides/5 +HDL-Cholesterol).

Immunohistochemistry

The paraffin embedded livers and adipose tissues, fixed in formalin saline 10%, were cut into 5 µm sections and mounted on positively charged slides for ERβ immunohistochemistry. Sections were dewaxed, rehydrated and autoclaved at 120^{o}c for 10 minutes in 10 Mm citrate buffer (PH 6). After washing with PBS endogenous peroxidase was blocked using 0.3% H2o2 in methanol (15 minutes). Slides were washed in PBS again and blocking was performed by adding blocking buffer and incubated for 30 minutes at room temperature. Primary antibody for ERβ (Cat. No. RB- 10658-R7, Thermo Scientific Co., UK) was added after dilution by PBS in a rate 1:10 and incubated for 30 minutes. Biotinylated polyvalent secondary antibody (Cat. No. 32230, Thermo Scientific Co., UK) was applied to tissue sections and co-incubated for 30 minutes after washing. The reaction was visualized by adding Metal Enhanced DAB Substrate according to (Bancroft and Cook, 1994).

Histopathology

Sections of 5µ thickness of livers that were fixed in 10% neutral buffered formalin were stained with haematoxylin and eosin and examined under light microscope according to (Bancroft and Gamble, 2007).

Quantification of IHC and adipocyte size

For quantitative analysis, the intensity of immunoreactive parts was used as a criterion of cellular activity after subtracting background noise. Measurement was done using an image analyzer (Image J program). From each slide of both experimental groups, 9 fields were randomly selected. The total field and immunohistochemial (IHC) stained areas were calculated then the %IHC stained area calculated as follow:

% IHC stained area = (IHC stained area)/ (Total area) X100%.

Adipocyte size in each experimental group was determined by the same program from 9 fields were randomly selected from each animal.

Oral glucose tolerance test

After overnight fasting, blood glucose levels of ovariectomized female rats were determined via glucometer (Accu-Chek Active, Germany). Then they were administered oral glucose solution (40%) 1g/ kg using gavage tube (Pederson *et al.*, 1998), then glucose levels were estimated by glucometer at 0 , 30 , 60 , 90, 120, 150 and 180 minutes.

Insulin tolerance test

Insulin tolerance test was performed 4 days after OGTT, using 0.75 U/kg human insulin (humulin) in 0.9% saline injected i.p after 30 min from oral glucose administration by same dose of OGTT. Blood glucose levels were estimated by glucometer at 0, 30, 60, 90 and 120 minutes.

Determination of plasma leptin and adiponectin levels

Plasma leptin and adiponectin concentrations were determined using commercial enzyme linked immunoassay rat assay kit (Code No. 27295, IBL, Japan and Cat. No. 22-ADPRT-E01, ALPCO diagnostics, USA), respectively according to manufacturer instruction.

Statistical analysis

All values were expressed as the mean ± SE. Differences among groups were determined by T test using SPSS program version 16.0. A value of $P < 0.05$ was considered to be statistically significant (Field, 2000).

RESULTS

The performed experiment demonstrated that dietary phytoestrogens significantly (P<0.05) lower the food intake in high dose group starting from 4th week of treatment 112.17±3.57 g/ week versus 126.33±3.8 g/ week in control group and till the end of experiment (Fig.1). Body weight gain was significantly (P<0.05) lower in high phytoestrogens-fed group starting from 5th week 0.87±0.38 g/week than control one 5.28±1.26 g/ week till the end of experiment.

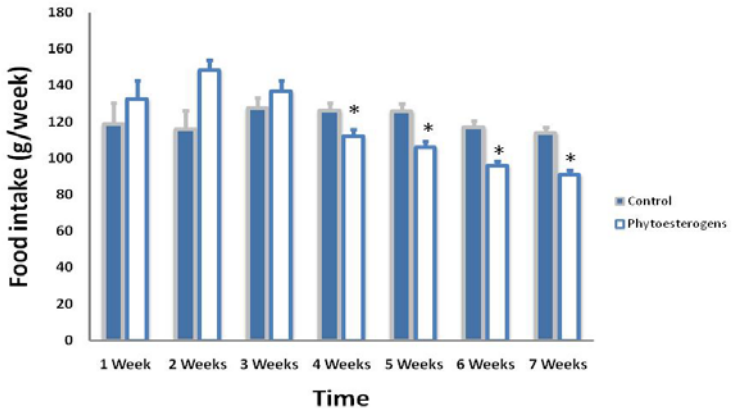

Fig. 1: Effect of dietary phytoestrogens on food intake g/ week of ovariectomized female rats

The abdominal, brown fat masses % and adipocytes diameter showed a significant (p<0.05) reduction in HF group than control group with values (Table 2).The values of lipid profile shown in Table (2) revealed that HDL was significantly (P<0.05) higher in treated group than control. While TG, TC and LDL showed significant decrease in its value in HF group than control. Adipocytes diameters showed the same trend while the expression of ERβ in adipocytes were significantly higher (P<0.05) in HF group than those of control one (Fig. 2 and Plate 1). Liver histopathological sections showed fatty infiltrations and steatosis in control group with sinusoidal dilatation while high phytoestrogens fed rats livers showed normal architecture without fat infiltration (Plate 2).

Table 2: Effect of dietary phytoestrogens on lipid profile mg/ dl, abdominal fat mass%, brown fat mass% adipocytes diameter (µm), plasma leptin levels and plasma adiponectin levels.

Parameters	*Control group*	*Phytoestrogen- treated group*
HDL mg / dl	10.42 ± 0.42	12.12 ± 0.48 *
TG mg / dl	115.21 ± 6.82	83.65 ± 8.49 *
TC mg / dl	66.74 ± 2.09	60.29 ± 1.88 *
LDL mg / dl	33.27±2.88	31.36±2.25*
Abdominal fat masses %	6.12±0.45	4.29±0.28*
Brown fat masses %	0.80±0.15	0.36±0.04*
Adipocytes diameter/ µm	74.16±15.94	32.45±3.452
Leptin levels ng/ml	200.6 ± 3.06	162.72 ± 3.015 *
Adiponectin levels ng/ml	4.005 ± 0.05	6.24 ± 0.17 *

(*) represents a significant difference between the control and treated groups, using Student Unpaired t-test ($p< 0.05$).

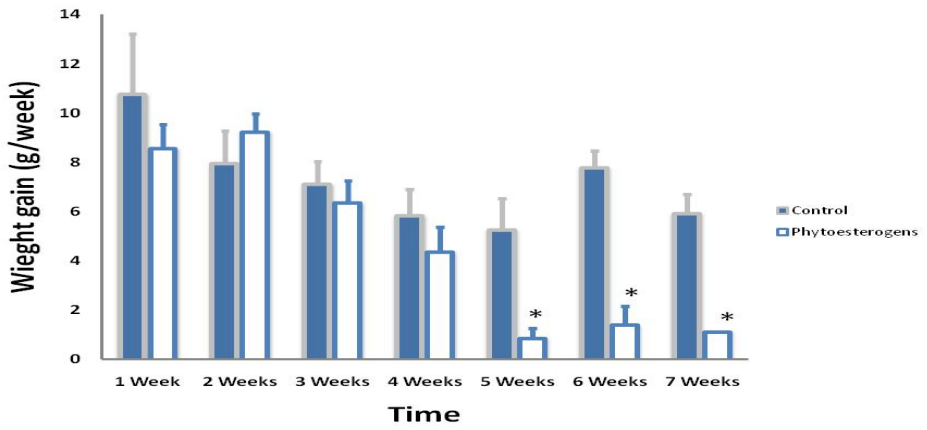

Fig. 2: Effect of dietary phytoestrogens on body weight gain g/ week of ovariectomized female rats

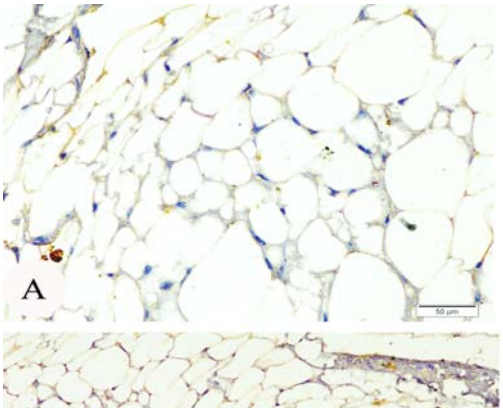

Plate 1: Immunostaining of abdominal fat masses A: Control, B: phytoesterogen-treated group. The plate showed that ERβ were localized predominantly within the cytoplasm. Phytoesterogen treatments produced a significant up regulation of ERβ expression and decrease in size in abdominal adipocytes relative to control.

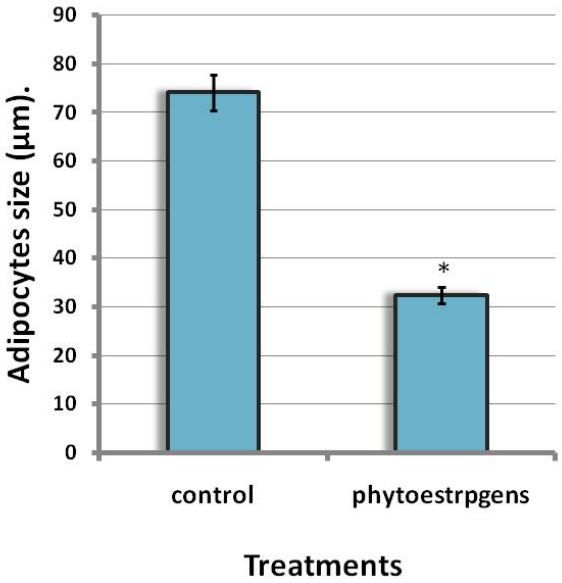

Fig. 3: Effects of phytoesterogens on ERβ expression in adipose tissue Change in adipocytes size compared with normal control group ($p< 0.05$).

Concerning blood glucose levels in oral glucose tolerance test (OGTT) no significant difference was observed between two tested groups However, it was

noticed from the curve it was noticed that phytoestrogens fed rats showed return to basal fasting glucose level after oral glucose tolerance while control group showed elevated glucose level than fasting one (Fig. 4). Insulin tolerance test (ITT) showed significant decrease in glucose level in phytoestrogens fed rats than control (Fig. 4).

Plasma leptin levels showed a significant ($P<0.05$) decrease in HF group than control while plasma adiponecin levels showed significant increase in HF group than control as shown in (Table 2).

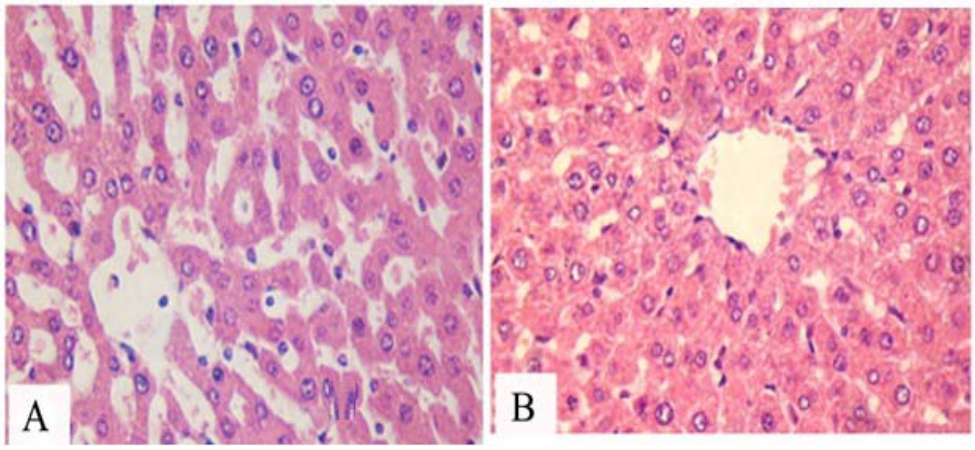

Plate 2: Representative photomicrographs showing liver histopathology of control group (*A*) and phytoesrogen-treated group (*B*) rats. Steatosis and dilatation of sinusoids were noted in control group [hematoxylin and eosin (H&E) stain; original magnification: ×20]

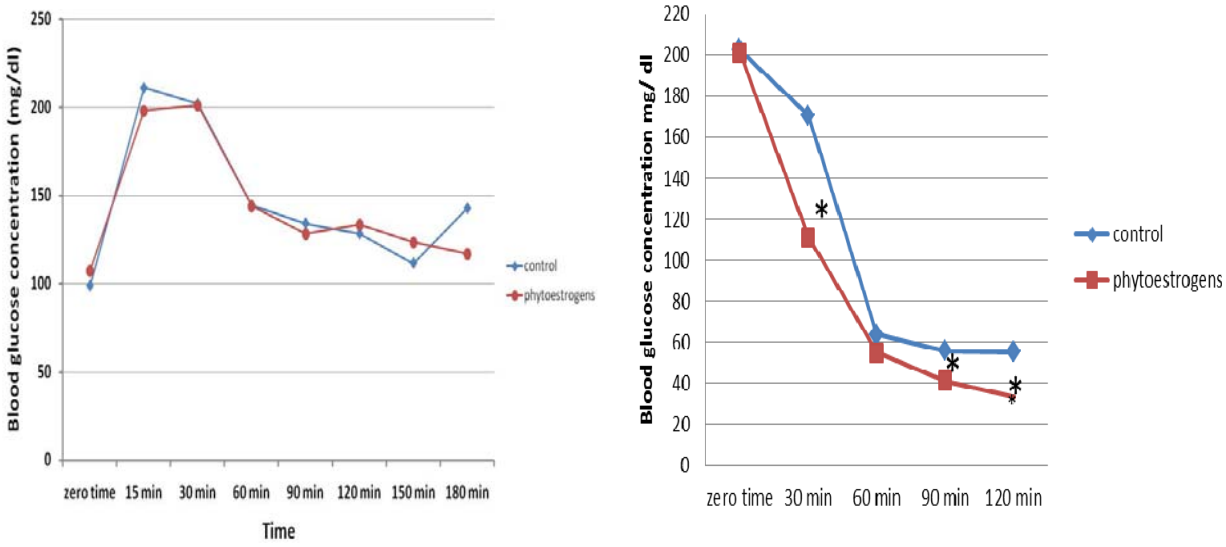

Fig. 4: Effect of phytoesterogens on OGTT (A) and ITT (B) of ovariectomized female rats.

DISCUSSION

The metabolic syndrome is characterized by obesity, insulin resistance, and a predisposition to hypertension, dyslipidemia, and type 2 diabetes. A common feature linking these metabolic abnormalities is the dysregulation of ERs expression in estrogen deprived condition and its consequent changes in energetic status, some adipokines (leptin and adiponectin), lipid profile, OGTT and ITT. The present study demonstrated the protective effects of *soy phytoestrogens* against the risks of obesity and metabolic syndrome in ovariectomized female rats under estrogen-deprivation conditions. Dietary soy phytoestrogens administered to ovariectomized female albino

rats affect food intake and substantially diminished the body weight gain in high phytoestrogens fed rats than control. These results are consistent with previous records (Kim *et al*., 2005 and Tolba 2013). Phytoestrogens are structurally similar to endogenous estrogens, they can act as a weak estrogen and bind to the ERs in various tissues (Naaz *et al*. 2003), thus reduction in feed intake may be due to the appetite repressing action of estrogen (Roy and Wade, 1975) as dietary phytostrogens decreased food intake and hence decreased body weight. The decrease implies that the estrogenic action of phytoestrogens is beneficial to body fat regulation due to the decreased level of leptin as observed in the current study that is produced from adipose tissue and influences hypothalamic neuropeptide Y (NPY) levels which regulates feeding behaviour and energy loss (Szkudelska *et al*., 2000 and Naaz *et al*., 2003). In the current study, the effect of soy phytoestrogens was manifested as decrease in abdominal, brown fat masses % and adipocytes diameter that could be attributed to apoptotic effect of phytoestrogens on adipocytes, suggesting that at least part of the weight loss is due to ablation of fat cells, which could result in better maintenance of weight loss (Kim *et al*., 2005). These results, are coincide with those recorded by Cederroth *et al*., (2007) and Cederroth *et al*., (2008) who found that mice fed dietary phytoestrogens were leaner due to increase their locomotor activity as observed in this study which may be due to preferential use of lipids as fuel source. These in vivo data are further supported by in vitro studies showing that genistein induced lipolysis and inhibited de novo lipid synthesis in 3T3-L1 adipocytes (Harmon & Harp 2001 and Harmon *et al*., 2002) and in rat adipocytes (Szkudelska *et al*., 2000). Phytoestrogens also affect fat growth and development, which is the main source of leptin, through peroxisome proliferator-activated receptors (PPARs) (Anderson *et al*., 2004) which is a major factor involved in *de novo* fatty acid synthesis, adipocyte differentiation, lipid accumulation, and adipocyte survival/maintenance (Jump *et al*., 2005).

Dietary phytoestrogens was also shown to have direct effects on lipid metabolism as it decreased TC, TG & LDL and increased HDL significantly ($P<0.05$) which are consistent with previous record of Kirk *et al*., 1998; Nogowski *et al*., 1998; Wangen *et al*., 2001, Uesugi *et al*., 2002 and Tolba, 2013. Because they affect lipid metabolism in liver and adipose tissue, decreasing triglycerides (Nogowski *et al*., 1998) that reflected by decrease in adipocytes diameter and decrease in liver fatty changes in treated group. These results suggests the hypolipidemic effect of phytoestrogens that are ascribed to their structural similarities to estradiol (E2) which acts predominantly via two distinct nuclear ERs, ERα and ERβ, defined as ligand-inducible transcription factors (Rosen *et al*., 2000). Dietary phytoestrogens increased adipocytes expression of ERβ that inhibits lipogenesis is primarily through decreasing expression levels and activity of lipoprotein lipase (LPL), an enzyme that regulates lipid uptake and filling of adipocytes (Misso *et al*., 2003, Naaz *et al*., 2003 and Heim *et al*., 2004). phytoestrogens might lower cholesterol levels by increasing LDL receptor activity, and the reduction in cholesterol may offer some protection against atherosclerosis (Kirk *et al*., 1998). Another explaination is that soy phytoestrogens decrease intestinal cholesterol absorption increase in bile acid excretion that mediate the lipid-lowering effect of soy protein (Greaves *et al*., 2000).

The observation that dietary phytoestrogens depressed significantly ($p<0.05$) plasma leptin levels, which is a mediator of long-term regulation of energy balance (Klok *et al*., 2007) than control group in combination with the depression in abdominal fat mass, brown fat mass% and adipocytes diameter allowed us to believe that this effect of phytoestrogens was due to its direct influence on adipocytes which

are the main source of leptin (Szkudelski *et al*., 2005). The effect of phytoestrogens especially genistein inhibit some enzymes in adipocytes substantially abates leptin secretion (Bradley and Cheatham 1999) in spite of unchanged expression of its gene (Szkudelski *et al*., 2005).

- In the present study dietary phytoestrogens doesn't affect OGTT significantly but it was noticed that glucose levels returned to its fasting level at the end of OGTT while in control the glucose levels were elevated above the fasting level at the end of OGTT. The significant improvement of ITT in treated group could be attributed to the elevated levels of plasma adiponectin that have receptors in liver and skeletal muscle, and the signaling through these receptors increases insulin sensitivity (Karastergiou and Mohamed- Ali 2010) and improves glucose tolerance (Wasim *et al*, 2006) by decreasing triglyceride content in muscle and liver (Yamauchi *et al*., 2001). Since genistein and daidzein have both been shown to bind to and activate PPARγ (Dang *et al*. 2003, Mezei *et al*, 2003 and Dang *et al*., 2004), it is likely that changes in insulin sensitivity could then be modified by adiponectin, which is increased in response to PPARγ agonists (Lihn *et al*., 2005 and Kadowaki *et al*., 2005). Moreover, adiponectin stimulates decreased gluconeogenesis, increased glucose uptake (Díez and Iglesias 2003 and Nedvídková *et al*., 2005), lipid catabolism (Vasseur *et al*.,2003), β-oxidation of fatty acids and triglyceride clearance (Nedvídková *et al*., 2005). The reduction of adiposity accompanied by decreased leptin and increased adipocytes expression of ERβ and ectopic fat deposition in liver with absence of steatosis in HF group that occurs with the elevated levels of adiponectin are in coincidence with the concept of Kadowaki *et al*., 2005 that exist the relationship between dietary phytoestrogens and ERs especially ERβ expression due to the preferential affinity of these compounds to ERβ (Kuiper *et al*., 1998) in regulation of body fat mass and glucose metabolism.

CONCLUSION

This study brought a new knowledge in understanding of the role of soy and its component phytoestrogens in the regulation of energy balance and obesity, it was appeared that these compounds may have more potent effects in prevention of some metabolic abnormalities that are part of the metabolic syndrome in estrogen depriving condition. The results of the current study showed that high dietary phytoestrogens interfere with adiposity by decreasing energy intake , increasing energy expenditure, reduction of lipid parameters (abdominal, brown fat mass, adipocytes diameter and lipid profile) with consequent reduction in plasma leptin levels while plasma adiponectin levels increased with improvement in insulin sensitivity and all this seem to follow the upregulation of ERβ expression in ovariectomized female rats.

REFERENCES

Ahima RS and Osei SY (2008): Adipokines in obesity. *Front Horm Res*., 36:182-97.

Anderson GL, Limacher M, Assaf AR, Bassford T and Beresford SA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, Chlebowski R, Curb D,, Gass M, Hays J, Heiss G, Hendrix S, Howard BV, Hsia J, Hubbell A, Jackson R, Johnson KC, Judd H, Kotchen JM, Kuller L, LaCroix AZ, Lane D, Langer RD, Lasser N, Lewis CE, Manson J, Margolis K, Ockene J, O'Sullivan MJ, Phillips L, Prentice RL, Ritenbaugh C, Robbins J, Rossouw JE, Sarto G, Stefanick ML, Van Horn L, Wactawski-Wende J, Wallace R and Wassertheil-Smoller S (2004): Effects of conjugated equine estrogen in postmenopausal women with hysterectomy. *J. Am. Med. Assco.;* 291(14): 1701-1712.

Atkinson C, Skor HE, Dawn Fitzgibbons E, Scholes D; Chen C, Wahala K, Schwartz SM and Lampe JWJ (2003): Urinary equol excretion in relation to 2-hydroxyestrone and 16-hydroxyestrone concentrations: an observational study of young to middle-aged women. *Steroid Biochem. Mol. Biol.*; 86(1):71–77.

Badman MK and Flier JS (2005): The gut and energy balance: visceral allies in the obesity wars. *Science*; 307(5717):1909–1914.

Bancroft JD and Cook HC (1994): Immunohistochemistry manual of histological techniques and their diagnostic applications. *W.B. Saunders Company 2nd ed:* 263-325.

Bancroft JD, Gamble M. (2007): Theory and Practice of Histological Techniques. 6th ed.

Bradley RL and Cheatham B (1999): Regulation of ob gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. *Diabetes;* 48(2):272-278.

Brennan AM and Mantzoros CS (2006): Drug Insight: the role of leptin in human physiology and pathophysiology--emerging clinical applications. *Nat. Clin. Pract. Endocrinol. Metab.*; 2(6):318-27.

Cederroth CR, Vinciguerra M, Gjinovci A, Kühne F, Klein M, Cederroth M, Caille D, Suter M, Neumann D, James R.W, Doerge DR, Wallimann T, Meda P, Foti, M, Rohner-Jeanrenaud F, Vassalli JD, and Nef, S (2008): Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. *Diabetes*; 57(5):1176-1185.

Cederroth CR, Vinciguerra M, Kühne F, Madani R, Doerge DR, Visser TJ, Foti M, Rohner-Jeanrenaud F, Vassalli JD, Nef S (2007): A phytoestrogen-rich diet increases energy expenditure and decreases adiposity in mice. *Environ Health Perspect*., 115 (10):1467–1473.

Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp R H, Brunzell JD and Kahn SE (2003): Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia*; 46(4): 459-469.

Cooke PS and Naaz A (2004): Role of estrogens in adipocyte development and function. *Exp. Biol. Med*.; 229(11):1127–1135.

Cooke PS, Heine PA, Taylor JA and Lubahn DB (2001): The role of estrogen and estrogen receptor-alpha in male adipose tissue. *Mol Cell Endocrinol*.; 178(1-2):147–154.

Dang ZC, Audinot V, Papapoulos SE, Boutin JA and Löwik CW (2003): Peroxisome proliferator-activated receptor gamma (PPARgamma) as a molecular target for the soy phytoestrogen genistein. *J Biol Chem.*; 78(2):962–967.

Dang ZC and Löwik CWGM (2004): The balance between concurrent activation of ERs and PPARs determine daidzein-induced osteogenesis and adipogenesis. *J Bone Min Res*.; 19(5):853–861.

Davidson MH and Rosenson RS (2009): Novel targets that affect high-density lipoprotein metabolism: the next frontier. *Am J Cardiol.*; 104(10 Suppl):52E-57E.

Díez JJ and Iglesias P (2003): The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol.* 148(3): 293–300.

Faughnan MS, Hawdon A, Ah-Singh E, Brown J, Millward DJ and Cassidy A (2004): Urinary isoflavone kinetics: the effect of age, gender, food matrix and chemical composition. *Br J Nutr.;* 91(4):567–574.

Field AP (2000): Discovering statistics using SPSS for windows: advanced techniques for the beginner. *Sage publications*; London.

Goodman-Gruen D and Kritz-Silverstein D (2003): Usual dietary isoflavone intake and body composition in postmenopausal women. *Menopause*; 10(5):427–432.

Greaves KA, Wilson MD, Rudel LL, Williams JK and Wagner JD (2000): Consumption of soy protein reduces cholesterol absorption compared to casein protein alone or

supplemented with an isoflavone extract or conjugated equine estrogen in ovariectomized cynomolgus monkeys. *J Nutr.;* 130(4): 820-826.

Harmon A, Patel Y and Harp J. (2002): Genistein inhibits CCAAT/enhancer-binding protein beta activity and 3T3-L1 adipogenesis by increasing C/EBP homologous protein expression. *Biochem J.*; 367(pt 1):203–8.

Harmon A and Harp J (2001): Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis. *Am J Physiol Cell Physiol.*; 280(4):C807–13.

Heim M, Frank O, Kampmann G, Sochocky N, Pennimpede T, Fuchs P, Hunziker W, Weber P, Martin I, Bendik I. (2004):The phytoestrogen genistein enhances osteogenesis and represses adipogenic differentiation of human primary bone marrow stromal cells. *Endocrinology*; 145(2):848–859.

Hong M, Lin MY, Huang JM, Baumeister P, Hakre S, Roy AL and Lee AS (2005): Transcriptional regulation of the Grp78 promoter by endoplasmic reticulum stress: role of TFII-I and its tyrosine phosphorylation. *J Biol Chem*.; 280(17):16821–16828.

Huang J, Nasr M, Kim Y and Matthews HR (1992): Genistein inhibits protein histidine kinase. *J Biol Chem.;* 267(22):15511–15515.

Hwang J, Wang J, Morazzoni P, Hodis HN and Sevanian A (2003): The phytoestrogen equol increases nitric oxide availability by inhibiting superoxide production: an antioxidant mechanism for cell-mediated LDL modification. *Free Radic Biol Med*.; 34(10):1271–1282.

Jayagopal V, Albertazzi P, Kilpatrick ES, Howarth EM, Jennings PE, Hepburn DA and Atkin SL (2002): Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. *Diabetes Care*; 25(10):1709–1714.

Jump DB, Botolin D, Wang Y, Xu J, Christian B and Demeure O. (2005). Fatty acid regulation of gene transcription. *J Nutr.;* 135(11):2503–2506. Kadowaki T and Yamauchi T (2005): Adiponectin and adiponectin receptors. *Endocr Rev.;* 26(3):439–51.

Karastergiou K and Mohamed-Ali V (2010): The autocrine and paracrine roles of adipokines. *Mol Cell Endocrinol.*; 318(1-2):69–78.

Kershaw EE and Flier JS (2004): Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab.*; 89(6):2548–2556.

Klok MD, Jakobsdottir S and Drent ML. (2007): The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes Rev*., 8(1): 21-34.

Kim S, Sohn I and Lee YS (2005): Hepatic gene expression profiles are altered by genistein supplementation in mice with diet-induced obesity. *J Nutr*.; 135(1):33–41.

Kirk EA, Sutherland P, Wang S, Chait A and LeBoeuf RC (1998): Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *J Nutr*.; 128(6):954–959.

Kritz-Silverstein D, Von Mühlen D, Barrett-Connor E and Bressel MA (2003): Isoflavones and cognitive function in older women: the SOy and Postmenopausal Health In Aging (SOPHIA) Study. *Menopause*; 10(3):196-202.

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der BB, Gustafsson JA. (1998): Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*; 139(10):4252–4263.

Lasota A and Danowska-Klonowska D (2004): Experimental osteoporosis- different methods of ovariectomy in female white rats. *Rocz Akad Med Bialymst.;* 49 (Suppl 1):129-31.

Lemay A, Dodin S, Kadri N, Jacques H and Forest JC (2002): flaxseed dietary supplement versus hormone replacement therapy in hypercholesterolemic menopausal women. *Obstet Gynecol.,* 100(3): 495–504.

Lihn AS, Pedersen SB and Richelsen B. (2005): Adiponectin: action, regulation and association to insulin sensitivity. *Obes Rev.;* 6(1):13–21.

Merz-Demlow BE, Duncan AM, Wangen KE, Xu X, Carr TP, Phipps WR and Kurzer MS (2000): Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *Am J Clin Nutr.;* 71(6): 1462–1469.

Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA and Shay N (2003): Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. *J Nutr.;* 133(5):1238–1243.

Misso ML, Murata Y, Boon WC, Jones ME, Britt KL and Simpson ER. (2003): Cellular and molecular characterization of the adipose phenotype of the aromatase-deficient mouse. *Endocrinology*; 144(4):1474–1480.

Naaz A, Yellayi S, Zakroczymski MA, Bunick D, Doerge DR and Lubahn DB, (2003): Helferich WG, Cooke PS. The soy isoflavone genistein decreases adipose deposition in mice. *Endocrinology*; 144(8):3315–3320.

Nedvídková J, Smitka K, Kopský V and Hainer V (2005): Adiponectin, an adipocyte-derived protein. *Physiol Res.;* 54 (2): 133–40.

Nogowski L, Mackowiak P, Kandulska K, Szkudelski T and Nowak KW (1998): Genistein-induced changes in lipid metabolism of ovariectomized rats. *Ann Nutr Metab.*; 42(6):360–366.

NRC, (1995): Nutrient requirements of laboratory animals. National Academic Paris. Washington. D. C. 4th Revised Edition.

Penza M, Montani C, Romani A, Vignolini P, Pampaloni B, Tanini A, Brandi ML, Alonso-Magdalena P, Nadal A, Ottobrini L, Parolini O, Bignotti E, Calza S, Maggi A, Grigolato PG and Di Lorenzo D (2006): Genistein Affects Adipose Tissue Deposition in a Dose- Dependent and Gender-Specific Manner. *Endocrinology*, 147(12):5740–5751.

Potter S, Baum J, Teng H, Stillman R, Shay N, Erdman-Jr J, (1998): Soy protein and isoflavones: their effects on blood lipids and one density in postmenopausal women. *Am J Clin Nutr*.; 68(6 suppl):1375S-1379S.

Pederson RA, White HA, Pauly DS, Robert P., Mclntosh CHS and Demuth H-U (1998): Improved Glucose Tolerance in Zucker Fatty Rats by Oral Administration of the Dipeptidyl Peptidase IV Inhibitor Isoleucine Thiazolidide. *Diabetes;* 47(8):1253-1258.

Ratna, W.N. (2002): Inhibition of estrogenic stimulation of gene expression by genistein. *Life Sci*.; 71(8):(865–877

Renaldi O, Pramono B, Sinorita H, Purnomo LB, Asdie RH and Asdie AH (2009): Hypoadiponectinemia: a risk factor for metabolic syndrome. *Acta Med Indones*; 41(1): 20–4.

Rosen ED, Walkey CJ, Puigserver P and Spiegelman BM (2000): Transcriptional regulation of adipogenesis. *Genes Dev*.; 14(11):1293–1307.

Roy EJ and Wade GN (1975). Role of estrogens in androgen-induced spontaneous activity in male rats. *J Comp Physiol Psychol*., 89(6):573-9.

Sanders TAB, Dean TS, Grainger D, Miller GJ and Wiseman H (2002): Moderate intakes of intact soy protein rich in isoflavones compared with ethanol-extracted soy protein increase HDL but do not influence transforming growth factor beta(1) concentrations and hemostatic risk factors for coronary heart disease in healthy subjects. *Am J Clin Nutr*.; 76(7):373–377.

Szkudelska K, Nogowski L and Szkudelski T (2000): Genistein affects lipogenesis and lipolysis in isolated rat adipocytes. *J Steroid Biochem Mol Biol.*, 75(4-5):265–71.

Szkudelska, T, Nogowski T, Pruszy´nska-Oszmałek E, Kaczmarek P and Szkudelska K (2005): Genistein restricts leptin secretion from rat adipocytes Journal of Steroid *Biochemistry & Molecular Biology*; 96(3-4):301-307

Teede, HJ, Dalais FS, Kotsopoulos D, Liang Y-L, Davis S and McGrath BP (2001): Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. J *Clin Endocrino. Metab*.; 86(7): 3053- 3060.

Tietz NW (1990): Clinical Guide to Laboratory Tests, Second Edition. Philadelphia, USA. W.B. Saunders Company: 554 – 556.

Tolba EAT (2013): Dietary Phytoestrogens Reduce the Leptin Level in Ovariectomized Female Rats. *IJCEBS* 1(3): 496-500.

Uesugi T, Fukui Y and Yamori Y. (2002): Beneficial effects of soybean isoflavone supplementation on bone metabolism and serum lipids in postmenopausal Japanese women: a four-week study. *J Am Coll Nutr*.; 21(2):97–102.

Ukkola O and Santaniemi M (2002): Adiponectin: a link between excess adiposity and associated comorbidities?. *J Mol Med* 80 (11): 696–702.

Vasseur F, Leprêtre F, Lacquemant C and Froguel P (2003): The genetics of adiponectin. *Curr Diab Rep.*, 3(2): 151–158.

Wangen, KE, Duncan AM, Xu X and Kurzer MS (2001): Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. *Am J Clin Nutr*.; 73(2):225–231.

Wasim H, Al-Daghri NM, Chetty R, McTernan PG, Barnett AH and Kumar S. (2006). Rela-tionship of serum adiponectin and resistin to glucose intolerance and fattopography in South-Asians. *Cardiovascular Diabetology*; 5:10.

Wesstfall RJ and Hauge SM (1948):The nutritive quality and the trypsin inhibitor content of soybean flour heated at various temperatures. *J Nutrition;* 35(3): 379-389.

Wilson VS, Bobseine K and Grey LE (2004): Development and characterization of a cell line that stably express on estrogen responsive luciferase reporter for detection of estrogen receptor agonist and antagonists. *Toxicol Sciences;* 81:(1) 69-77.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P and Kadowaki T (2001):The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med.,* 7(8):941-6.