# SERUM ADIPONECTIN AND IRISIN LEVELS IN OVARIECTOMIZED OSTEOPOROTIC RAT MODEL

By

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## ABSTRACT

**Background:** Post-menopausal osteoporosis (PMOP) is the most common bone disease in females characterized by decreased bone mineral density (BMD). However, the exact pathogenesis remains unclear.

**Objective:** Investigating the relationship between serum levels of adiponectin and irisin, and their relation to BMD in ovariectomy induced osteoporosis rat model.

**Material and Methods:** Three equal groups of adult female albino rats (n=15) were used; i.e. control, sham operated and ovariectomized (OVX) groups. Nine weeks after ovariectomy, serum analysis of adiponectin, irisin, FSH, estradiol, $Ca^{++}$, phosphorus (P), alkaline phosphatase (ALP), glucose, and insulin were estimated. Final BMI and HOMA-IR were calculated. Bone BMD measurements (dry and ash femur weight, bone $Ca^{++}$ and P together with femur histopathological examination) were done.

**Results:** In OVX osteoporotic rat model, while serum levels of adiponectin significantly elevated and negatively correlated with BMD, serum irisin levels significantly reduced, and showed significant positive correlation with BMD. Changes in OVX adiponectin and irisin levels were significantly associated with the elevated insulin resistance. However, they were not association with FSH or estradiol levels.

**Conclusion**: OVX induced osteoporosis was associated with a significant increase in adiponectin levels, and decrease in irisin levels which are associated with changes in insulin resistance rather than sex hormones. It can be hypothesized that the exact causative of PMOP extends beyond pituitary; ovarian axis to be metabolic, muscle and adiposity cross talks which needs more detailed investigations.

**Key words**: Ovariectomy, osteoporosis, adiponectin, irisin, rats.

## INTRODUCTION

Osteoporosis is one of senile degenerative diseases affects more than 200 million individuals worldwide. It is a growing major public health problem that characterized by reduction of the bone mineral density (BMD), disruption of the bone micro-architecture that increases the possibility of fractures and osteopathology **(Aaseth et al., 2012, Appelman-Dijkstra & Papapoulos 2014, Emkey & Epstein, 2014, Xin et al., 2014, and Iliou et al., 2015).**

Postmenopausal osteoporosis (PMOP) is recognized to be secondary to alterations in the pituitary-bone axis, whereas postmenopausal reduced estrogen is claimed as the main pathogenesis **(Seibel et al., 2006).**

Adipose tissue secretes a variety of biologically active molecules which are named adipocytokines **(won Muhlen et al., 2007).** This may regulate bone metabolism and be involved in osteoporosis pathophysiology. Cumulative evidence has shown that there is an association between BMD and fat mass

(Ho-Pham et al., 2014 and Mohiti-Ardekani et al., 2014)

Adiponectin is one of the adipocyto-kines, highly expressed in visceral and bone marrow fat deposits and abundantly present in plasma (Araneta et al., 2009). So, it has been proposed to share in the regulation of energy homeostasis and insulin sensitivity (Williams et al., 2009 and Jiang et al., 2011). Previous studies have shown crosstalk between adiponectin and bone metabolism (Kanazawa et al., 2007 and Mohiti-Ardekani et al., 2014).

Richards et al. (2007) have demons-trated that adiponectin exerts an indepen-dent negative effect on BMD. However; other investigators have shown that there was no independent relationship between adiponectin and BMD (Basurto et al., 2009).

Adiponectin is significantly associated with BMD at total body, lumbar spine, total hip, and total forearm in post-menopausal women. This association is not related to fat mass or other hormonal factors studied (Zhang et al., 2010). Therefore, it was suggested that adipo-nectin is an independent predictor of BMD in post-menopausal women (Zhang et al., 2010 and Tohidi et al., 2012). Moreover, Mohiti-Ardekani et al. (2014) showed that serum adiponectin had a significant negative correlation with BMD of the femoral neck and lumbar spine in osteoporotic patients.

It has been widely shown that increased muscle mass, measured as lean body mass, is related to increased BMD and a reduction in vertebral fracture risk. In addition, brown adipose tissue volume is known to be a positive predictor of femoral bone structure and correlates positively with thigh muscle suggesting that sarcopenia is related to osteoporosis (Kaji, 2013 and Bredella et al., 2014). Irisin is a myokine and adipokine induced in exercise and stimulates adipose tissue browning (Bostr?m et al., 2012 and Roca-Rivada et al., 2013).

The circulating irisin levels have been associated with the incidence fractures in postmenopausal women with low bone mass (Anastasilakis et al., 2014). Also, Palermo et al. (2015) have confirmed an inverse correlation between irisin levels and vertebral fragility fractures in postmenopausal women, but no significant correlation was found with BMD or lean mass. Furthermore, it has been shown that irisin enhances the differentiation of bone marrow stromal cells into osteoblasts in vitro (Colaianni et al., 2014).

The present study aimed to evaluate serum levels of adiponectin and irisin in postmenopausal osteoporosis rat model (OVX), and investigate whether serum adiponectin and/or irisin levels were associated with BMD and bone turnover biochemical markers or not.

## MATERIALS AND METHODS

Animals: Forty five healthy adult female albino wistar rats weighing 198 ± 8.5gm were obtained from the animal house in Faculty of Veterinary Medicine, Zagazig University. Animals were kept in nine steel wire cages (40 x 28 x18 Cm. 5 rats /cage) under hygienic conditions w in animal house of Faculty of Medicine, Zagazig University. All animals received care in accordance with the guide to the care and use of experimental animals of Institute of Laboratory Animal Resources (1996). The experimental

protocol was approved by the Institutional Review Board and research ethics committee of Faculty of Medicine, Zagazig University (IRB). Animals were fed standard chow and had free access to water. The rats were accommodated to animal house conditions for one week before the experiments going on. Rats were divided randomly into three equal groups, control sham operated and OVX groups.

**Rat model of osteoporosis**

Bilateral ovariectomy and sham ovari-ectomy were done according to the methods of **Nishizawa et al. (2002) and Gui et al. (2004).**

At the end of experiments (Nine weeks after ovariectomy), rats were weighed, and BMI were calculated according to the equation: body weight (gm)/length$^2$ (nose to anus length) (cm$^2$) (**Novelli et al., 2008**), and then blood and tissue samples were obtained.

**Blood sampling:** All animals were fasted overnight, anesthetized by diethyl ether; blood was collected from retro-orbital venous plexus and allowed to clot for 2 hours at room temperature before serum was separated by centrifugation of clotted blood at 3000 rpm for 20 minutes. The separated serum was stored at -80° C until used for further analysis.

Rats were sacrificed by cervical dis-location and then both femurs were obtained.

**Serum was analyzed for** irisin levels according to **Bostr?m et al. (2012)** using rat irisin ELISA rat kit (Catalog # K4761-100), biovision, Milpitas Blvd., Milpitas, CA 95035 USA, Adiponectin levels according to **Arita et al. (1999)** using rat

Adiponectin ELISA rat kit (Cat. # EZRADP-61K), Follicle stimulating hormone (FSH), Estradiol (E2), and Progesterone (PROG) according to the method of **Tietz (1995)** using ELISA rat kits: BC-1029 and BC-1115, respectively, BioCheck Inc 323 Vintage Park Dr. Foster City, CA 94404, serum glucose level according to **Tietz (1995)** using glucose enzymatic- liquizyme rat kits (Biotechno-logy, Egypt), insulin level according to **Temple et al. (1992)** using KAP1251-INS-EASIA rat Kits (BioSource Europe S.A., Belgium). Homeostatic model assessment of insulin resistance index (HOMA-IR) based on serum insulin level (?IU/ml) and serum glucose level (mg/dl) was calculated according to the formula described by Matthews **et al. (1985)**: HOMA- IR = fasting serum glucose (mg/dl) X fasting serum insulin (?IU/ml) /405, serum calcium (Ca$^{++}$) levels by colorimetric method according to Gindler et al. (1972) using kits supplied by Bio-diagnostic Co. (Cairo, Egypt), serum phosphorus (P) levels by colorimetric method according to **Goldenberg, (1966**) using kits supplied by Bio-diagnostic Co. (Cairo, Egypt), and serum alkaline phosphates (ALP) levels according to **Belfied and Goldberg (1971).**

**Bone density** was determined according to **Doster et al. (1969).** Calcium and phosphorus levels in bones were detected by colorimetric methods using spectronic 21 according to **Garcia-Contreras et al. (2000**).

**Bone histolopathological examination:**

Left femur was dissected from each rat, fixed and placed in 10% formaline solution for one day (**Raab et al., 1991).** Tissues were processed in ascending

grades of alcohol, cleaned in xylol and embedded in paraffin blocks. Four microns sections were cut on a standard rotatory microtome and stained by Heamatoxylin and Eosin (H and E) stain as described by **Bancroft and Cook (1984).**

**Statistical analysis:** Results were presented as mean ± standard deviation (SD). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, IL, United States). Repeated measures of analysis of variance (ANOVA) were applied followed by the Stude nt-least significant deference (LSD), post hoc test to compare means of each two different groups. Pearson's correlation analysis was performed to screen potential relations between serum levels of adiponectin and irisin and all measured parameters. For all statistical tests done, P value < 0.05 was considered to be statistically significant.

## RESULTS

No significant difference was detected between sham operated group and negative control group in all parameters measured (P>0.05). OVX group showed significant elevation of the mean values of serum levels of adiponectin (12.14±2.32 n/mL), FSH (8.63±1.00 mIU/ml) and alkaline phosphatase "ALP" (179.19± 9.62 I$\mu$/ L) in comparison to those of sham operated group (10.82±1.32, 5.02±0.70 and 142.56±7.38 respectively) (P<0.05, P<0.001 and P<0.001 respectively). Serum levels of irisin (0.75±0.15 ?g/ml) and estradiol (14.36 ±2.11 pg/ml) in OVX group were significantly lower when compared to that of sham operated group (1.42±0.17 and 26.29±4.61 mg/dL

respectively) (P<0.001). Moreover, among the three studied groups, serum levels of $Ca^{++}$ (10.51± 0.38, 10.52±0.37 and 10.21± 0.51 respectively) and P (5.39±0.68, 5.37± 0.71 and 5.09±0.77 mg/dL, respectively) showed non-significant difference (P>0.05- Table 1).

No significant difference could be detected between sham operated group and negative control group in all parameters measured (P>0.05). OVX group revealed significant increase in the mean values of BMI (0.64±0.56 gm/$Cm^2$), serum insulin (13.59±2.05 $\mu$IU/mL) and calculated HOMA-IR (5.03± 0.63) when compared to those of sham operated group (0.49±0.02gm/$Cm^2$, 7.87±1.36,$\mu$IU/mL, 2.13± 0.25 respectively) (P< 0.001, P<0.001 and P<0.01 respectively). However, BMD parameters of OVX group were significantly lower (dry weight 334.87±43.64, mg/femur, ash weight 203.13±29.06, mg/femur, bone $Ca^{++}$ 91.28±8.4 mg/femur and bone P 31.54±1.93 mg/femur) than those of sham operated group (504.86±40.06mg/femur, 309.8±37.03 mg/femur, 146.24±11.89 mg/ femur, 39.48±4.47 mg/femur respectively) (P<0.001). However, serum glucose levels (mg/dL) did not show any significant changes among the three studied groups (76.32±8.33, 74.19±9.61 and 81.32±9.66 respectively) (P>0.05- Table 2).

**Pearson's correlation analysis** between both of serum adiponectin and serum irisin with the measured parameters in OVX group (table 3) showed a significant negative correlation between the serum adiponectin levels and BMI (r = -0.616, P=0.009), insulin (r = -0.639, P=0.007), HOMA-IR (-0.701, P=0.001), serum ALP (r= -0.773, P<0.001) (Table 3). Dry femur weight was r = -0.981 (P<0.001), ash

femur weight was r = -0.703 (P<0.001) (figure 1A), bone $Ca^{++}$ was r= -0.694 (P = 0.001) and bone P (r = -0.893 (P<0.001) (figure 1B). However, no significant association, could be detected between serum adiponectin and serum FSH (r = 0.395, P=0.054) or serum estradiol (r = 0.358, P=0.058), while serum irisin showed significant negative correlation with BMI (r= -0.653, P=0.004), serum insulin (r= -0.676, P=0.001) and HOMA-IR (r= -0.733, P=0.001), it revealed significant positive correlation with serum ALP (r= 0.901, P< 0.001), dry femur weight (r= 0.982, P<0.001), ash femur weight ( r= 0.863, P<0.001) (figure 2A), bone $Ca^{++}$ (r = 0.826, P<0.001) and bone

P (r= 0.875, P<0.001) (figure 2B). However, no significant association, could be detected between serum irisin and serum FSH (r= 0.383, P=0.059) or serum estradiol (r= 0.372, P=0.055).

**Histopathological examination:**

The photomicrographs of control and sham operated groups showed normal compact bone tissues with normal haversian system, the haversian canal surrounded by normal osteoblasts (**Figure 3 and 4**). The OVX groups showing thin atrophic bone trabeculae with wide marrow spaces (**Figure 5**).

**Table (1): Statistical analysis of serum levels of irisin (?g/ml), adiponectin (ng/ml), FSH (μIU/ml), E2 (pg/ml), $Ca^{++}$ (mg/dL), P (mg/dL), and ALP activity (Iu/ L) in the three studied groups.**

| Groups / Parameters | Control | Sham operated | OVX |
|---|---|---|---|
| Adiponectin (ng/ml) | 10.84±1.36 | 10.82±1.32, P= 0.97[a] | 12.14±2.32, P= 0.046 [a], P=0.043[b] |
| Irisin (?g/ml) | 1.41±0.16 | 1.42±0.17, P= 0.91[a] | 0.75±0.15, P<0.001 [a,b] |
| FSH (mIU/ml) | 5.03±0.74 | 5.02±0.70, P= 0.99[a] | 8.63±1.00, P<0.001[a,b] |
| Estradiol (pg/ml) | 27.29±4.88 | 26.29 ±4.61, P= 0.92[a] | 14.36±2.11,P<0.001[a,b] |
| $Ca^{++}$ (mg/dL) | 10.51± 0.38 | 10.52±0.37, P= 0.99[a] | 10.21±0.51, P= 0.59[a], P = 0.060[b] |
| P (mg/dL) | 5.39±0.68 | 5.37 ± 0.71, P= 0.92[a] | 5.09±0.77, P= 0.25[a], P = 0.29[b] |
| ALP (Iμ/ L) | 143.44±7.39 | 142.56±7.38, P= 0.96[a] | 179.19± 9.62, P<0.001 [a,b] |

a = p value of significance versus control, b = p value of significance versus sham operated group.

**Table (2): Statistical analysis of calculated BMI (gm/Cm$^2$), serum glucose (mg/dL), serum insulin (μIU/mL) and calculated HOMA-IR and bone mineral density (BMD) parameters in the three studied groups.**

| Groups / Parameters | Control | Sham operated | OVX |
|---|---|---|---|
| BMI (gm/Cm$^2$) | 0.48±0.02 | 0.49±0.02 P= 0.62[a] | 0.64±0.56, P=0.000 [a,b] |
| Glucose (mg/dL) | 76.32±8.33 | 74.19±9.61, P= 0.73[a] | 81.32±9.66, P= 0.55[a,b] |
| Insulin (μIU/mL) | 8.07±1.19 | 7.87±1.36 , P= 0.32[a] | 13.59±2.05, P=0.001 [a,b] |
| HOMA-IR | 2.02± 0.13 | 2.13± 0.25 , P= 0.54[a] | 5.03± 0.63, P= 0.01 [a,b] |
| Dry wt (mg/femur) | 510.80±39.00 | 504.86±40.06, P= 0.69[a] | 334.87±43.64, P<0.001 [a,b] |
| Ash wt (mg/femur) | 310.00±36.0 | 309.8±37.03, P= 0.80[a] | 203.13±29.06, P<0.001 [a,b] |
| Bone $Ca^{++}$ (mg/femur) | 147.83±10.59 | 146.24±11.89, P= 0.68[a] | 91.28±8.4, P<0.001 [a,b] |
| Bone P (mg/femur) | 40.67±3.54 | 39.48±4.47, P= 0.35[a] | 31.54±1.93, P<0.001 [a,b] |

a = p value of significance versus negative control, b = p value of significance versus sham operated group, P< 0.05 is considered significant.

**Table (3): Pearson's correlation analysis between serum adiponectin (ng/ml) and serum irisin (?g/ml) levels with calculated BMI (gm/Cm2), serum insulin (μIU/mL), calculated HOM A-IR, serum FSH (μIU/ml), serum E2 (pg/ml), Serum ALP (Iμ/ L), dry weight (mg/femur), ash weight (mg/femur), bone Ca++ (mg/femur), bone Phosphorus (P) (mg/femur) in OVX group.**

| Sera \ Parameters | Adeponectin (ng/ml) | | Irisin (?g/ml) | |
|---|---|---|---|---|
| | r | P | r | P |
| BMI (gm/Cm$^2$) | -0.616 | P= 0.009 | -0.653 | P= 0.004 |
| Insulin (mIU/mL) | -0.639 | P= 0.007 | -0.687 | P= 0.001 |
| HOMA-IR | -0.701 | P= 0.001 | -0.733 | P<0.01 |
| FSH (mIU/ml) | 0.395 | P= 0.054 | 0.383 | P= 0.059 |
| E2 (pg/ml) | -0.358 | P= 0.058 | -0.372 | P= 0.055 |
| Serum ALP (Iμ/ L) | -0.773 | P< 0.001 | 0.901 | P<0.001 |
| Dry weight (mg/femur) | -0.981 | P<0.001 | 0.982 | P<0.001 |
| Ash weight (mg/femur) | -0.703 | P<0.001 | 0.863 | P<0.001 |
| Bone Ca$^{++}$ (mg/femur) | -0.694 | P<0.001 | 0.826 | P<0.001 |
| Bone P (mg/femur) | -0.893 | P<0.001 | 0.875 | P<0.001 |
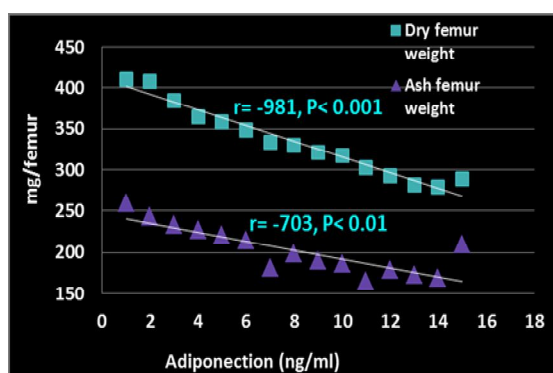


**Figure (1A):** Pearson's correlation analysis between both of dry femur weight (mg/femur) and ash femur weight (mg/femur) with serum adiponectin levels (ng/ml) in OVX group.
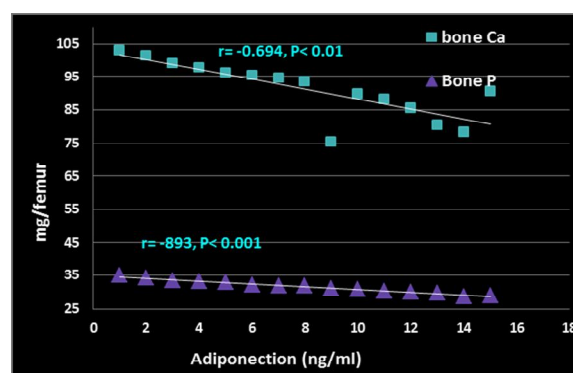


**Figure (1B):** Pearson's correlation analysis between both of bone calcium content (mg/femur) and bone phosphorus content (mg/femur) with serum adiponectin levels (ng/ml) in OVX group.
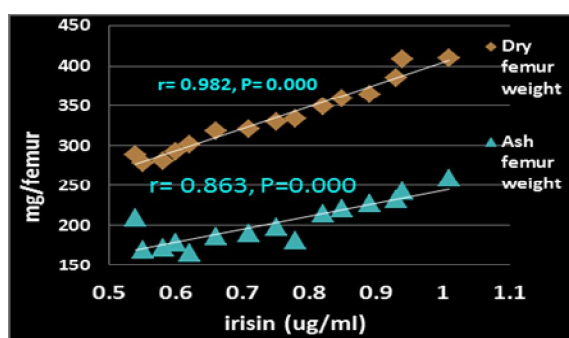


**Figure (2A):** Pearson's correlation analysis between both of dry femur weight (mg/femur) and ash femur weight (mg/femur) with serum irisin levels (μg/ml) in OVX group.
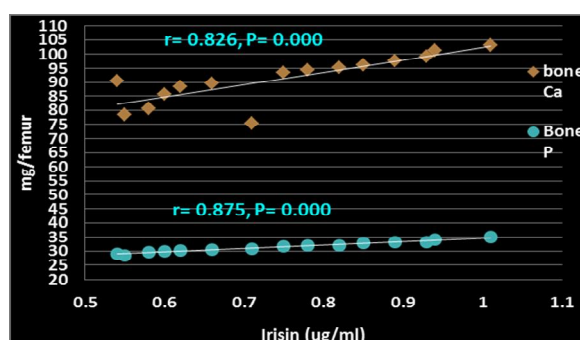


**Figure (2B):** Pearson's correlation analysis between both of bone calcium content (mg/femur) and bone phosphorus content (mg/femur) with serum irisin levels (μg/ml) in OVX group.
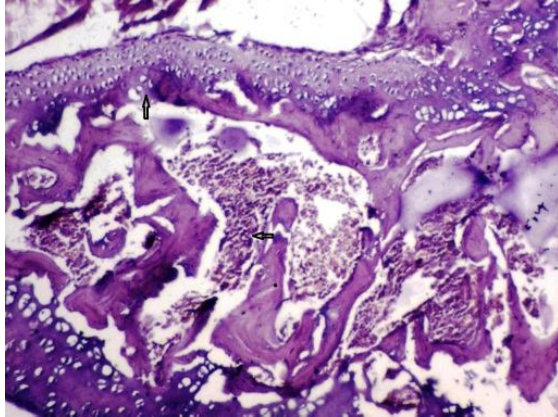
**Figure (3):** Photomicrograph of compact bone tissue of control group formed of normal haversian system with haversian canal surrounded by normal osteoblasts (H and E, X -400).
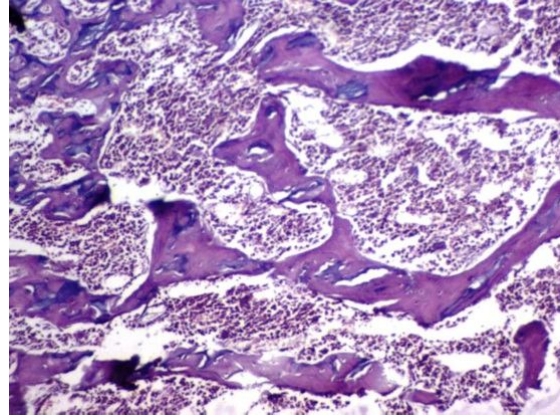


**Figure (4):** Photomicrograph of compact bone tissue of sham operated group formed of normal haversian system with haversian canal surrounded by normal osteoblasts (H and E, X-400).
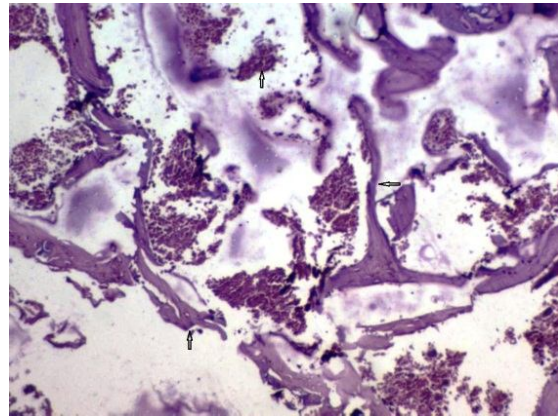


**Figure (5):** Photomicrograph of compact bone tissue of OVX group showing atrophic bone trabeculae with wide marrow spaces containing bone marrow cells (H and E, X-400).

# DISCUSSION

In the present results, the statistical comparison between shame operated and control groups revealed no significant difference in all studied parameters. The present study revealed that BMD decreased in OVX group in the form of a significant decrease in the mean values of femur dry weight, ash weight and calcium and phosphorus contents of bony ashes accompanied by a significant increase in serum alkaline phosphatase activity in comparison with that of the control and sham operated groups, These diagnostic criteria of decrease in BMD are in line with the results of OVX rat model of osteoporosis done by **Griffith et al. (2010)** and **Ce et al. (2014a).**

Serum FSH levels were significantly increased, while estradiol significantly decreased in OVX rats compared with that of sham operated group and a significant negative correlation between FSH serum levels with BMD parameters was noted. These results come in accordance with previous studies of **Gallagher et al. (2010), Cheung et al. (2011),** and **Garcia-Martin et al. (2012)** who reported that serum FSH levels were significantly increased and negatively correlated with BMD in PMOP independently of estrogen. In addition **Wang et al. (2015)** concluded that FSH may play an

important role in the acceleration of bone loss in postmenopausal women and increase osteoclastogenesis in vitro. **Sun et al. (2006)** proved t hat female mice lacking either FSHβ or the FSH receptor were resistant to bone loss despite hypogonadism. **Sowers et al. (2013)** and **Imai (2014)** also stated that the rate of bone mass loss during perimenopause is greater than that in postmenopause**,** whereas estrogen serum levels during perimenopause are normal (**Sowers et al., 2006).** However, inconsistent studies of **Seibel et al. (2006), Ritter et al. (2008) and Gourlay et al. (2012)** indicated that FSH does not appear to modulate bone mass regulation in vivo and does not act directly on osteoclastogenesis in vitro.

**Rouach et al. (2011)** reported that estrogen deficiency is the dominant cause of bone loss in OVX rats, but FSH may be closely related to hypogonadal bone loss. **Liu et al. (2010a &b)** found that FSH can aggravate alveolar bone loss by FSH receptor activation independently of estrogen. In addition, FSH inhibitors prevent bone loss in ovariectomized rats. However, the etiology of postmenopausal osteoporosis extends beyond pituitary and ovarian sex hormones.

Current results showed a significant elevation in serum levels of adiponectin in OVX rats in comparison to control and sham operated groups. Moreover, these elevated level of adiponectin showed significant negative correlation with BMI, insulin levels and HOMA-IR. However, it failed to be correlated with FSH or estradiol. The present finding was in line with the results of **Ce et al. (2014b)** who proved an increase in serum adiponectin and visceral adiposity in OVX rats. The

significant increase in serum adiponectin levels could be explained by a physiological response to preserve systemic insulin sensitivity (**Ainslie et al., 2001)**, as ovariectomy in the present study was accompanied by significant elevation in HOMA-IR (decrease in insulin sensitivity). In the same context **Saengsirisuwan et al.** (**2009)** reported metabolic alterations mimicking features of the insulin resistance syndrome in ovariectomy rats**.**

**Siri and Ginsberg** (**2003**) reported significant increase in insulin resistance in human after ovariectomy, Moreover, **Prasannarong et al.** (**2012**) stated that prolonged ovariectomy resulted in dyslipidemia, impaired glucose tolerance and reduced insulin-stimulated skeletal muscle glucose transport. It has also been reported that adiponectin levels were significantly higher in late postmeno-pausal women (**Moorthy et al., 2004)**. Interestingly, estrogen treatment after ovariectomy protects against fatty liver and may improve pathway selective insulin resistance (**Zhu et al., 2013**), and is able to attenuate the increase of serum adiponectin levels in OVX rats (**Ce et al., 2014b)**. However, no significant association between adiponectin levels and the elevated serum levels FSH or decreased serum levels of estradiol could be detected the present work.

Regarding the association between adiponectin and BMD, the present research found significant negative correlation between the elevated serum adiponectin levels and decreased BMD parameters in OVX group, and these data were consistent with those of **Mohiti-Ardekani et al. (2014**) and **Mpalaris et**

al. **(2016)** who found that serum adiponectin level was high in osteoporosis and negatively correlated with lumbar and femur BMD. While more studies showed a negative correlation between adiponectin and BMD (**Richards et al., 2007, Ealey et al., 2008, Pang et al., 2008, Zhang et al., 2010 and Tohidi et al., 2012**), some researcher reported no correlation or positive correlations (**Zhong et al., 2005 and Parm et al., 2011**).

The negative association between adiponectin and BMD in osteoporosis could be an indicator for bone resorption activity. **Berner et al. (2004)** proved the presence of adiponectin receptor on osteoblasts. Also, **Luo et al. (2006)** showed that adiponectin enhances the receptor activator of nuclear factor- kappa B ligand (RANKL) expression so, indirectly increasing osteoclast formation and inhibiting osteoprotegerin (OPG) production in osteoblasts**.** OPG prevents RANKL from binding to receptor activator of nuclear factor kappa B (RANK) and results in the suppression of osteoclastogenesis (**Secchiero et al., 2006**).

Adiponectin could be considered as one of the body responses to increased bone resorption induced by insulin resistance. Insulin is an anabolic hormone, which acts on bone through insulin receptors (IRs) expressed by osteoblasts IRS-1 and IRs-2 (insulin-like substrate). Stimulation of IRs-1 affects bone turnover, while stimulation of IRs-2 shifts the balance towards resorption. Insulin stimulates osteoblast proliferation, promotes collagen synthesis, and

increases glucose uptake (**Nyman et al., 2011**).

In T2DM, hyperinsulinism coupled with insulin resistance has a negative effect on BMD (**R?kel et al., 2008, Arikan et al., 2012 and Hamann et al., 2012**). The mirror image of adiponectin levels and HOMA-IR has been proved in human, monkey, and rodents that adiponectin is an insulin-sensitizing hormone (**Kubota et al., 2006** and **Nway et al., 2016**). In support with the hyposthesis of compensatory role of adiponectin is the study of **Williams et al. (2009)** who reported that adiponectin enhances human osteoblast proliferation and differentiation (osteoblastogenesis) in cultured osteoblasts. Furthermore, the introduction of recombinant adiponectin to human osteoblasts has been demonstrated to induce osteoblast formation, as well as stimulate the osteoclast RANKL pathway, while inhibiting its decoy receptor OPG. Thus, adiponectin may be exerting its effect on bone metabolism through the RANKL pathway in osteoporosis (**Atalay et al., 2012; Ochoa et al., 2012 and Mohiti-Ardekani et al., 2014**).

**Kajimura et al. (2013)** reported that adiponectin has the unusual ability of being able to regulate the same function in two opposite manners depending on where it acts and what it opposes. **Shinoda et al. (2006)** suggested that circulating adiponectin induces a positive action through the indirect pathway via enhancement of the insulin signaling and a negative action through the direct pathway.

Regarding serum irisin levels in OVX rats, it was significantly lower as com-

pared with that of sham operated and control groups and negatively correlated with BMI and HOMA-IR. However, no significant association between serum irisin levels and FSH or estradiol levels could be detected. Our results were confirmed by **Anastasilakis et al. (2014)** who have shown lower serum irisin levels in postmenopausal women with previous osteoporotic fractures. **Aubertin-Leheudre et al. (2008)** speculated that low irisin levels may result from sarcopenic (muscle weakness) obesity and decreased muscle strength. **Moreno-Navarrete et al. (2013)** stated that FNDC5 (fibronectin [type 3] domain-containing [protein] 5) gene expression in muscle significantly decreased in association with T2DM obese participants. Thus, circulating irisin levels were negatively associated with obesity and insulin resistance. Furthermore, **Li et al. (2015)** proved that metformin which is known by improving insulin sensitivity, up-regulated intracellular FDNC5 mRNA/protein expression and promoted irisin release.

There was a significant positive correlation between irisin serum levels and dry femur weight, ash femur weight and bone $Ca^{++}$ level (BMD parameters). This result was in agreement with that of **Singhal et al. (2014)** who found a significant positive correlation between irisin levels and some bone quality parameters (volumetric bone mineral density, stiffness and failure load) measured by high resolution peripheral quantitative CT and finite element analysis. Moreover, **Gao et al. (2016)** found a positive correlation between serum irisin levels and bone mineral density in the control group, and a negative correlation in the polycystic ovary group after BMI and age adjusted.

In addition, **Colaianni et al. (2015)** observed significant increases in cortical bone mass and strength of male mice bone after injection with recombinant irisin. **Ustün et al. (2016)** stated that postmenopausal osteoporosis was associated with decreased levels of circulating irisin and chemerin. **Palermo et al. (2015)** also detected an inverse correlation between irisin levels and vertebral fragility fractures. However; they failed to found any significant correlation with BMD, lean mass or daily physical activity.

In vitro**,** study of **Zhang et al. (2013)** revealed that irisin increased bone trabecular density and cortical thickness in mice by activating osteoblasts differentiation via the Wnt/β-catenin pathway in osteoblastic MC3T3-E1 cells. In addition, irisin inhibits osteoclast differentiation via suppression RANKL/nuclear factor of activated T cells (**Kawao and Kaji, 2015).**

Also, **Colaianni et al. (2014)** proved that irisin directly targets osteoblast enhancing their proliferation and differentiation. In continuation**, Colaianni and Grano (2015)** reported that irisin exerts its effect on osteoblast lineage by enhancing differentiation and activity of bone-forming cells through the increase in activating expression of transcription factor 4. Moreover, irisin increases the expression of osteoblastic transcription regulators such as Runt-related transcription factor-2, osterix/sp7; and osteoblast differentiation markers, including alkaline phosphatase, collagen type 1 alpha-1, osteocalcin, and osteopontin. It also increases ALP activity and calcium deposition in cultured osteoblast (**Qiao et al., 2016).**

## CONCLUSION

Ovariectomy induced osteoporosis is associated with a significant increase in serum levels of adiponectin and decrease in serum levels of irisin. While serum levels of adiponectin were negatively associated with BMD, serum irisin levels showed a significant positive correlation with BMD. These changes in serum adiponectin and irisin levels may be related to metabolic rather than sex hormones disturbance. It can be hypothesized that the exact causative of postmenopausal osteoporosis extends beyond pituitary and ovarian sex hormones to be metabolic, muscle and adipose tissue cross talks which need more detailed investigations.

## ACKNOWLEDGMENT

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# مستويات الأديبونيكتين والأيرزين في مصل دم نموذج هشاشة العظام المحدث بإستئصال المبيضين في الجرذان

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**خلفية البحث:** تمثل هشاشة العظام بعد إنقطاع الطمث مرض العظام الأكثر شيوعا في الإناث ، ويتسم هذا المرض بإنخفاض كثافة المعادن في العظام ومع ذلك لا تزال الأسباب الدقيقة غير واضحة.

**الهدف من البحث:** التعرف على العلاقة بين مستويات الاديبونيكتين والأيرزين في مصل دم نموذج هشاشة العظام المحدث باستئصال المبيضين بالجرذان وعلاقتهما بدلائل كثافة العظام.

**طرق و مواد البحث:** تم استخدام ثلاث مجموعات متساوية من الجرذان الإناث البالغات البيضاء (العدد = ١٥) و المجموعات هي . المجموعة الضابطة والمجموعة الصورية والمجموعة المستأصلة المبايض.

وبعد ٩ أسابيع من إستئصال المبايض، تم أخذ عينات الدم من حزمة الأوردة خلف العين و فصل مصل الدم منها. و قد تم قياس مستويات هرمون الأديبونكتين و هرمون الأيرزين وهرمون تحفيز جريبات المبيض و هرمون الاستراديول والجلوكوز وهرمون الإنسولين و مستوي الكالسيوم والفوسفور ،وإنزيم الفوسفاتيز القلوي. وقد تم حساب كل من مؤشر كتلة الجسم و معدل المقاومة للإنسولين. وقد تم قياس دلائل هشاشة العظام (الوزن الجاف ووزن الرماد لعظم الفخذ ومستوي الكالسيوم و الفوسفور في العظام) جنبا إلى جنب مع فحص الأنسجة في عظم الفخذ.

**نتائج البحث:** بالنظر إلي نموذج هشاشة العظام بالجرذان، في حين أن مستويات مصل اديبونيكتين كانت مرتفعة بشكل ملحوظ وله دلالة إحصائية وتتناسب عكسيا مع كثافة المعادن بالعظام، إلا أن مستويات الإيرزين قد انخفضت بالدليل الإحصائي، وأظهرت ارتباطاً إيجابياً بدلالة إحصائية مع كثافة المعادن بالعظام. و قد إرتبطت التغيرات في مستويات الأديبونيكتين و الإيريسين بشكل كبير مع مقاومة الإنسولين المرتفعة. ومع ذلك، فإنها لم تظهر إرتباطا مع مستويات هرمون تحفيز جريبات المبيض أو هرمون الإستراديول.

**الإستنتاج:** ترتبط هشاشة العظام التي يسببها استئصال المبيضين في زيادة كبيرة في مستويات أديبونيكتين وإنخفاض في مستويات إيرزين. و قد ارتبطت هذه التغيرات مع التغيرات في مقاومة للإنسولين وليس الهرمونات الجنسية. ويمكن الإفتراض أن المسبب الدقيق لهشاشة العظام فيما بعد إنقطاع الطمس يتجاوز محور الغدة النخاميةـ المبيض بأن يكون تبادل علاقات فيما بين التمثيل الغذائي والعضلات والسمنة والذي يحتاج إلي تحقيقات أكثر تفصيلا.