Modulation of Fetuin-A by Estrogen and Caloric Restriction in Experimental Rat Model of Menopause

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Abstract

Background and objectives: Menopause is accompanied by higher prevalence of atherogenic lipid profiles, central obesity, and insulin resistance. Hepatic steatosis is frequent in postmenopausal women. Fetuin-A level is strongly related to liver fat content. Its role in menopausal metabolic derangements is largely unknown. We aimed to investigate the relation between fetuin-A and the development of menopausal metabolic complications related to obesity and if caloric restriction/estrogen supplementation could alleviate these abnormalities. **Methods:** Adult female Wistar rats were either bilaterally ovariectomized (OVX) (30 rats) or sham operated (sham group, n=10 rats). OVX rats were randomly subdivided into ovariectomized group (OVX), ovariectomized group treated with 17-β estradiol (OVX+E_2), and ovariectomized- caloric restricted group (OVX+CR) (n=10/group). Thirteen weeks following surgery, serum levels of glucose, insulin, lipids, fetuin-A and adiponectin were estimated. Weight of visceral fat and triacylglycerol (TAG) content of liver were assessed. **Results:** Serum fetuin-A level was increased following ovariectomy (113%, P<0.001). This was associated with increased visceral adiposity, abnormal lipid profile, insulin resistance, hypoadiponectinaemia and excessive accumulation of hepatic triacylglycerol (TAG). However, estrogen (E_2) supplementation/caloric restriction (CR) significantly suppressed elevated Fetuin-A (by 41.9%, P<0.001 and 31.2%, P<0.001, respectively). In addition, CR was equally effective as E_2 replacement in reversing metabolic abnormalities. **Conclusion:** Fetuin-A could be one of the factors contributing to the development of metabolic complications related to menopausal obesity. Caloric restriction alone can ameliorate those complications without the hazardous effects of estrogen replacement; possibly, through inhibition of serum fetuin-A level.

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INTRODUCTION

Menopause is a time of great physical, psychological and biochemical changes for women. The decrease in estrogen levels in menopausal women is accompanied by alterations in energy homeostasis that result in loss of subcutaneous fat and an increase in visceral fat. [1] The increasing incidence of overweight and obesity in menopausal women is a public health concern. [2] The reasons behind obesity in menopause are not fully understood. Some researchers refer it to the absence of estrogens. [3] Psychosocial factors may also contribute to menopausal obesity. However, the exact causes for obesity require further clarification.

Previous studies observed that daily food intake is increased in ovariectomized rats. [4] On the contrary, estrogen replacement therapy (ERT) was found to modulate the expression or activity of molecules involved in orexigenic/anorexigenic actions in hypothalamus, such as pro-opio-melano-cotrin (POMC), Melanin-concentrating hormone (MCH) and neuropeptide Y (NPY) and result in decreased appetite. [5, 6, 7]

Menopausal women are at higher risks of metabolic dysfunction predisposing to cardiovascular diseases, type 2 diabetes mellitus (T2DM)), and metabolic syndrome. Higher prevalence of atherogenic lipid profiles, central obesity, hyperinsulinemia, and insulin resistance are common metabolic disorders. [8] Moreover, Nonalcoholic Fatty Liver Disease (NAFLD) is frequent in postmenopausal women. [9] Alterations in body composition, fat distribution and/or hormonal or metabolic changes that occur following menopause may influence the development and progression of NAFLD, [10] that ranges from simple steatosis to necro-inflammation (steatohepatitis). [11]

As ovariectomy enhances appetite, [4] the consequences of ovarian hormone deprivation in obesity and insulin resistance are aggravated by the effects of increased caloric intake. [12] However, the benefits of caloric restriction in ovariectomized rat model of menopause have not been fully determined.

Fetuin-A is an abundant serum protein produced predominantly in the liver. [13] Fetuin-A was originally described as a growth factor in fetal calf serum [14] and was later identified as an important inhibitor of ectopic calcification. [15] Among its pleotropic actions, fetuin-A was observed to inhibit autophosphorylation of insulin receptors through suppression of tyrosine kinase activity. [16] Thus, it may contribute to insulin resistance. [17] Furthermore, Fetuin-A was shown to induce the expression of some inflammatory cytokines associated with the metabolic syndrome and inhibit the expression of adiponectin, an adipokine with anti-inflammatory and insulin sensitizing actions. [18]

While previous data have suggested a link between high fetuin-A levels and the metabolic syndrome, and reported a significant correlation between plasma levels of fetuin-A and liver fat content in subjects with increased risk of the metabolic syndrome, [19] the role of fetuin-A in menopausal metabolic derangements is largely unknown.

Taken together, the aim of this study was to investigate the hypothesis of fetuin-A being one of the factors contributing to the development of menopausal metabolic complications related to obesity. In addition, we aimed to explore whether

those metabolic disorders can be simply prevented by CR and maintained body weight, or ERT is crucial in preventing them, with special concern to the impact of CR and ERT on fetuin-A level in surgically induced menopause in rats.

MATERIALS AND METHODS

Experimental animals

Adult female Wistar rats weighing 220–250 g at the beginning of the experiment were used. Animals were housed under constant temperature ($25 \pm 2°C$) and maintained in a 12 h light/dark cycle. All animals received care according to ethical guidelines of Alexandria University on laboratory animals and the protocol was approved by the Faculty of Medicine, Alexandria University Ethics Committee.

Surgery and ovariectomy

Rats were allowed to acclimatize for 1 week prior to surgery. Then, rats were anesthetized by intraperitoneal *(ip)* sodium thiopental (30 mg/kg) and both ovaries were removed according to previously described technique. [20] Sham operated animals were subjected to same surgical procedure except that ovaries were gently manipulated but not excised. All rats were injected with gentamycin (5 mg/kg, *ip*) during surgery and then daily for one week. After surgery, rats were housed individually and observed for some hours to allow recovery and then re-grouped in their home cages.

Experimental groups

Rats in the experimental groups were either bilaterally ovariectomized (30 rats) or sham operated (**sham group,** n=10 rats). Following surgery, all rats were allowed to recover for one week. Then, OVX animals were randomly assigned into three groups (10 rats each):

ovariectomized group (OVX), ovariectomized group treated with 17-β estradiol (OVX+E$_2$), and **ovariectomized- caloric restricted group (OVX+CR).** Ovariectomized rats in the OVX+E$_2$ group were treated with 30 µg/kg body weight/ day of 17-ß-estradiol-3-benzoate; dissolved in 0.01% dimethylsulfoxide (DMSO) and administrated subcutaneously *(SC)*, [21] while rats in OVX, OVX+CR and sham groups received daily SC dose of the vehicle. Except OVX+CR group, rats were fed standard laboratory chow ad libitum; the amount of food consumed by each rat per day was determined and OVX+CR rats were given 65% of the amount of chow consumed by the OVX group in the previous day. [22] Treatments started one week after surgery and continued for 12 weeks.

Estimation of body weight gain

All rats were weighed one week after surgery, before treatments supplementation, to determine baseline weights. Then, 12 weeks later, rats were weighed again to obtain end weight. Weight gain was calculated for each rat as the difference between the two weights.

Sampling and biochemical analysis

Thirteen weeks following surgery (12 weeks of treatments), animals were fasted for 12 hours, and then euthanized by an overdose of thiopental. Blood samples were collected by cardiac puncture, allowed to clot and then centrifuged at 3000 rpm for 15 min to obtain serum. Fasting glucose was immediately measured by enzymatic colorimetric method. The remaining serum fractions were stored at -80°C until further biochemical analysis

Measurement of serum fetuin-A and adiponectin levels

Fetuin-A and adiponectin were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (WKEA MED Supplies Corp, NY, USA), on a spectrophotometric reader at a wavelength of 450 nm.

Assessment of insulin level and calculation of insulin resistance

Fasting serum insulin level was quantified using Rat Insulin ELISA Kit following the manufacturer's instructions (WKEA MED Supplies Corp, NY, USA). Insulin resistance (IR) of individual rats was evaluated using the previously validated homeostasis model assessment (HOMA-IR) index. [23] The used formula was as follows:

[HOMA-IR] = fasting serum glucose (mg/dL) × fasting serum insulin (μU/mL)/405.

Assessment of lipid profile

Serum total cholesterol (TC), and triglycerides (TG) were assayed using enzymatic colorimetric method. High density lipoprotein- cholesterol (HDL-C) was analyzed using NS Biotec HDL-precipitating reagent. Low density lipoprotein-cholesterol (LDL-C) was calculated using Friedewald formula: LDL-C (mg/dl) = TC – HDL-C – (TG/5). [24]

Weighing of visceral fat

Immediately after blood sampling, the abdominal cavity was opened; the perinephric, mesenteric, and retroperitoneal fat pads were removed and weighed. The sum of their weights was calculated as an indicator for visceral fat accumulation. [25]

Estimation of liver triacylglycerol (TAG)

The liver was immediately removed, washed by iced phosphate buffered saline (PBS) and stored at -80° C. After total lipids extraction using a chloroform/methyl alcohol (2:1) mixture, according to Folch et al.. [26] As a measure for hepatic fat content, triacylglycerol (TAG) in liver homogenate was measured spectrophotometrically (at 490 nm) using an Enzymatic Triglyceride Kit (Biovision), following the manufacturer's instructions

Statistical analysis:

Values are presented as mean ± SEM. Groups were compared by one way ANOVA, followed when significant, by post-hoc with least significant difference test for pair-wise comparisons. Pearson coefficient correlations were performed between different variables. Statistical analysis was performed using the Statistical Package for Social Sciences 12.0 for Windows (SPSS, Chicago. IL). $P < 0.05$ was considered statistically significant.

RESULTS

Body weight gain and weight of visceral fat

Twelve weeks following ovariectomy, OVX group had significant higher weight gain (P<0.001) and visceral fat accumulation (P<0.001), in comparison to sham counterparts. On the contrary, administration of 17β-estradiol or caloric restricted diet significantly declined weight gain (P<0.001 for both groups), and visceral fat buildup (P<0.001 for both groups) versus OVX rats. (Figure 1a, b)

Serum fetuin-A level

As shown in figure-2a, ovariectomy significantly increased serum fetuin-A levels by 113 % versus sham operated group (P<0.001). However, OVX+E$_2$ and OVX+CR groups presented significant lower fetuin-A (by 41.9 %, P<0.001 and 31.2 %, P<0.001, respectively) as compared to

OVX group, yet, they were not significant versus each other (P=0.093).

Serum Adiponectin level

When compared to sham group, OVX rats showed significant decrease (by 38.9 %, P<0.001) in serum Adiponectin. On the contrary, 17 β-estradiol administration and caloric restriction for 12 weeks significantly increased Adiponectin levels by 54 %, (P=0.003) and 44.8 %, (P=0.013), respectively, versus OVX group. (figure 2b)

Glycemia, insulinemia, and insulin resistance

Table-1 reveals that OVX rats showed significant higher values of glucose (P<0.001), and insulin (P=0.022), with subsequent higher insulin resistance marker (HOMA-IR) (P<0.001) versus sham group. Estrogen therapy had succeeded to reduce the increased HOMA-IR (P<0.001), observed in OVX rats through reduction of both glucose (P=0.014) and insulin (P<0.001) levels. Nevertheless, food restriction also reduced insulin resistance marker (P<0.001) through reduction in insulin (P<0.001), but not glucose (P=0.246) levels, when compared to OVX group.

Lipid profile

As demonstrated in table-1, OVX group exhibited significant higher values of serum TC, TG and LDL-C (P<0.001 for all), and significant lower value of HDL-C (P=0.002) versus sham operated controls. Estrogen administration for 12 weeks significantly reversed these changes; reduced TC TG and LDL-C, and increased HDL-C (all P<0.001, versus OVX group). Interestingly, the

improvement of lipid profile by caloric restricted diet in OVX rats was equivalent to estrogen supplementation.

Liver TAG

Hepatic lipid accumulation, assessed by liver TAG content, was significantly increased in OVX rats (P<0.001) versus sham controls. However, OVX+E_2 and OVX+CR groups showed significant decrease in liver TAG in comparison with OVX rats (P<0.001 in both groups). (Figure 3)

Correlations between serum fetuin-A and other variables:

In the pooled groups, significant high positive correlations between serum fetuin-A levels and adiposity related measurements; weight gain (r=0.70, P<0.001), weight of visceral fat (r=0.57, P<0.001) and liver TAG content (r=0.64, P<0.001) were observed. Moreover, fetuin-A level showed significant positive correlation with glycaemia, insulinaemia, and HOMA-IR (r=0.43, p=0.006; r=0.33, p=0.035 and r=0.48, p=0.002, respectively). In addition, significant positive correlation was observed between fetuin-A with TC (r=0.47, p=0.002), TG (r=0.45, P= 0.003) as well as LDL-C (r=0.52, p<0.001). On the other hand, fetuin-A was negatively related with HDL-C (r=-0.46, p=0.003). Importantly, the negative correlation between serum fetuin-A and adiponectin was significant (r=-0.46, p=0.003). (Table 2 and Fig. 4).
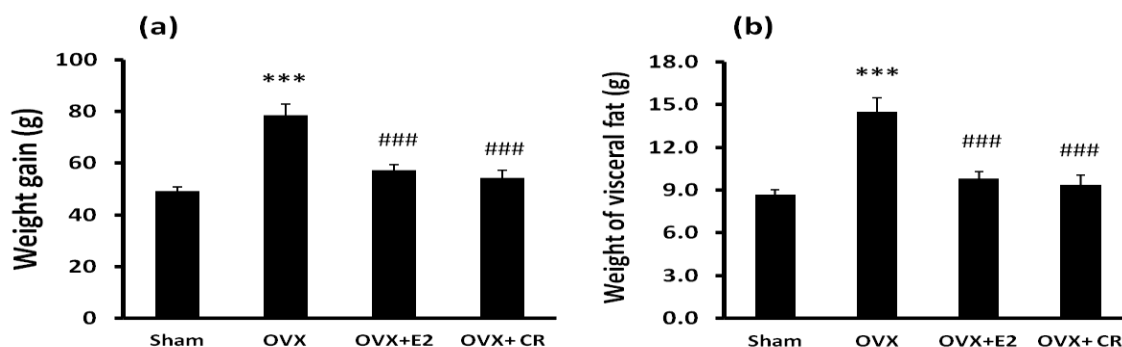
Fig. 1. Effect of 17B – estradiol administration and calorie restricted diet on (a) weight gain and (b) weight of visceral fat in ovariectomized (OVX) rats. Data are represented as means ± SEM of ten animals. * p<0.05, ** p<0.01, *** p<0.001 versus sham; # p<0.05, ## p<0.01, ###p<0.001 versus OVX group. P value is calculated by least significant difference test.
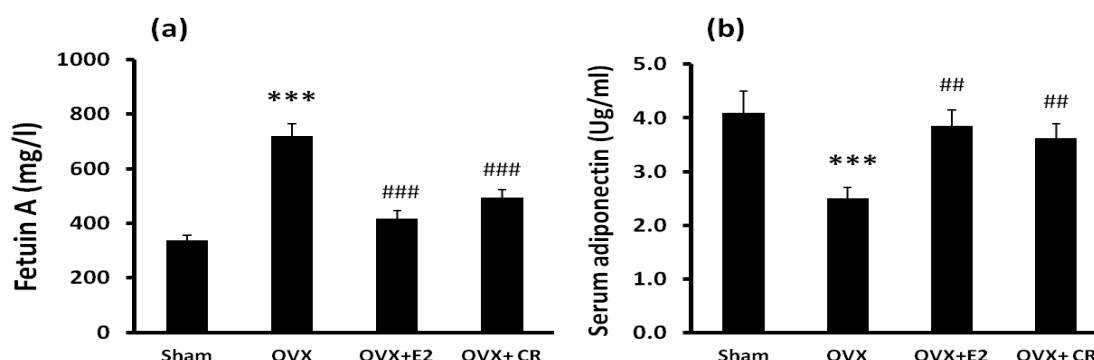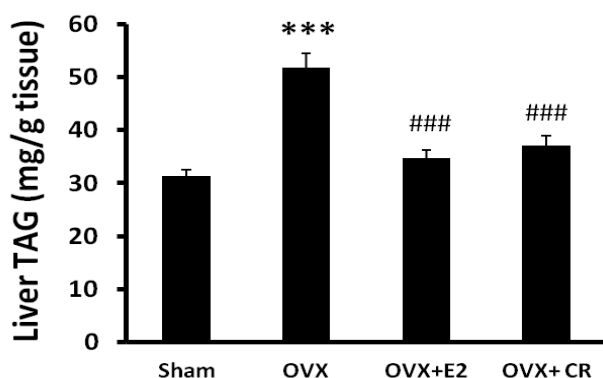

Fig. 2. Effect of 17B – estradiol administration and calorie restricted diet on (a) serum fetuin-A and (b) adiponectin levels in ovariectomized (OVX) rats. Data are represented as mean ± SEM of ten animals. Statistical differences were assessed by one-way ANOVA followed, when significant, by a least-significant-difference post-hoc test for pair-wise comparisons. * p<0.05, ** p<0.01, *** p<0.001 versus sham; # p<0.05, ## p<0.01, ###p<0.001 versus OVX group. P value is calculated by least significant difference test

Table 1: Effects of 17 β - estradiol administration or caloric restriction on glucose and insulin levels, insulin resistance and lipid profile of ovariectomized rats.

	Sham	OVX	OVX+E_2	OVX+CR	P ANOVA
Fasting glucose (mg/dl)	88.8 ± 2.7	117.9 ± 3.0***	104.7 ± 4.5 [#]	111.9 ± 4.0	<0.001
Fasting Insulin (IU/ml)	6.13 ± 0.67	7.73 ± 0.48*	5.11 ± 0.343 [###]	5.3 ± 0.31 [###]	<0.001
HOMA-IR	1.35 ± 0.16	2.27 ± 0.19***	1.31 ± 0.09 [###]	1.48 ± 0.12 [###]	<0.001
Total- C (mg/dl)	86.7 ± 3.5	116.9 ± 6.4***	87.8 ± 2.8 [###]	81.9 ± 4.1 [###]	<0.001
Triglycerides (TG) (mg/dl)	60.9 ± 2.5	79.7 ± 4.4***	54.9 ± 3.8 [###]	58.5 ± 3.1 [###]	<0.001
HDL-C (mg/dl)	39.8 ± 2.1	30.3 ± 1.7**	41.5 ± 2.6 [###]	41.7 ± 1.7 [###]	<0.001
LDL-C (mg/dl)	34.7 ± 4.7	70.7 ± 6.7***	35.3 ± 2.2 [###]	28.5 ± 4.0 [###]	<0.001

Data are represented as mean ± SEM of 10 rats per group; OVX, ovariectomized; E_2, 17 β-estradiol; CR, calorie restricted. HOMA-IR, homeostatic model assessment of insulin resistance index; C, cholesterol; HDL-C, high density lipoprotein- cholesterol; LDL-C, low density lipoprotein- cholesterol. Statistical differences were assessed by one-way ANOVA followed, when significant, by a least-significant-difference post-hoc test for pair-wise comparisons. *P<0.05, **P<0.01, ***P≤0.001 versus sham, #P<0.05, ##P<0.01, ###P≤0.001versus OVX group. P value is calculated by least significant difference test.

Fig. 3. Effect of 17B – estradiol administration and calorie restricted diet on liver triacylglycerol (TAG) in ovariectomized (OVX) rats. Data are represented as mean ± SEM of ten animals. Statistical differences were assessed by one-way ANOVA followed, when significant, by a least-significant-difference post-hoc test for pair-wise comparisons. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ versus sham; # $p<0.05$, ## $p<0.01$, ###$p<0.001$ versus OVX group. P value is calculated by least significant difference test.

Table 2: Correlation matrix of the studied variables

		Fetuin A (Ug/ml)	Weight gain (g)	Visceral fat (g)	Liver TAG (mg/g tissue)	Serum adiponectin (Ug/ml)	Fasting glucose (mg/dl)	Serum insulin (µIU/l)	HOMA - IR	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)
Weight gain (g)	r	0.70										
	p	<0.001										
Visceral fat weight (g)	r	0.57	0.65									
	p	<0.001	<0.001									
Liver TAG (mg/g tissue)	r	0.64	0.64	0.49								
	p	<0.001	<0.001	0.001								
Serum adiponectin (Ug/ml)	r	-0.46	-0.36	-0.29	-0.40							
	p	0.003	0.021	0.072	0.01							
Fasting glucose (mg/dl)	r	0.43	0.39	0.52	0.38	-0.32						
	p	0.006	0.012	<0.001	0.016	0.045						
Serum insulin (µIU/l)	r	0.33	0.30	0.35	0.50	-0.46	0.26					
	p	0.035	0.058	0.025	0.001	0.003	0.106					
HOMA-IR	r	0.48	0.43	0.52	0.58	-0.51	0.63	0.90				
	p	0.002	0.006	0.001	<0.001	0.001	<0.001	<0.001				
TC (mg/dl)	r	0.47	0.46	0.65	0.55	-0.24	0.22	0.50	0.54			
	p	0.002	0.003	<0.001	<0.001	0.140	0.174	0.001	<0.001			
TG (mg/dl)	r	0.45	0.49	0.41	0.61	-0.28	0.26	0.58	0.60	0.55		
	p	0.003	0.001	0.008	<0.001	0.083	0.106	<0.001	<0.001	<0.001		
HDL-C (mg/dl)	r	-0.46	-0.57	-0.46	-0.42	0.18	-0.15	-0.19	-0.25	-0.39	0.46	
	p	0.003	<0.001	0.003	0.007	0.262	0.341	0.243	0.122	0.013	0.003	
LDL-C (mg/dl)	r	0.52	0.54	0.68	0.55	-0.24	0.21	0.43	0.48	0.95	0.48	-0.65
	p	0.001	<0.001	<0.001	<0.001	0.141	0.186	0.005	0.002	<0.001	0.002	<0.001

TAG, triacylglycerol; HOMA-IR, homeostatic model assessment of insulin resistance index; C, cholesterol; HDL-C, high density lipoprotein- cholesterol; LDL-C, low density lipoprotein- cholesterol; r, Pearson's correlation coefficient for pooled data (n=40).

DISCUSSION

Through the menopausal effects, women can increase total body fat content, favoring the central body fat distribution. [27] The reasons behind increasing abdominal obesity in menopausal women are not clear. Probably, estrogen deficiency is a major contributing factor. [3]

Fetuin-A was previously implicated in insulin resistance and metabolic complications in different obesity-associated disorders. [19] Therefore, it was

noteworthy to investigate its possible role in ovariectomized rat model of menopause.

The relevant findings in this study included increased serum fetuin-A level after 13 weeks of ovariectomy. This was associated with increased visceral adiposity, abnormal lipid profile, insulin resistance, hypoadiponectinaemia and excessive accumulation of hepatic TAG. However, chronic CR was equally effective as E_2 supplementation in ameliorating these metabolic derangements in OVX rats; decreased central fat content, improved lipid profile and insulin resistance and reduced TAG deposition in the liver. These changes were associated with reduced fetuin-A and increased adiponectin levels.

In the present study, a significant increase in the weight gain and visceral fat accumulation in the ovariectomized group compared to the sham operated group was found. This was expected since previous reports demonstrated that estrogen deficiency causes not only fat redistribution with decreasing the subcutaneous fat and increasing the harmful visceral fat [28] but also increases appetite causing hyperphagia by modulating the activity of molecules involved in regulation of food intake. [29] Consistent with previous results [30], estrogen supplementation for 12 weeks significantly decreased weight gain and visceral fat of OVX rats; this was probably due to the reversed effect of estrogen deficiency by estrogen replacement. In a review on estrogen regulation of adipose tissue functions, it was reported that estrogen reduces adiposity by promoting the use of lipid as a fuel through activation of pathways that promote fat oxidation in muscle, inhibition of lipogenesis in

adipose tissue, liver, and muscle and improvement of adipocyte lipolysis rates. [31]

The CR group reported similar findings regarding the weight gain and visceral fat as E_2 supplemented group, with no significant difference versus each other. The probable reason is that, with caloric restriction we controlled the amount of calories introduced to the rats, so the hyperphagia- induced obesity is checked. Since weight loss generally induce preferential mobilization of visceral fat, [32] weight of abdominal fat pads was reduced by CR. Same observations were encountered by Meyer et al., who demonstrated that caloric restriction with or without exercise can reverse lipid deposition in visceral and hepatic tissues in overweight subjects. [33] This suggests that menopausal obesity could be simply prevented by caloric restriction

Alterations in body composition, fat distribution and/or hormonal or metabolic changes that occur following menopause may influence the development and progression of NAFLD. [10] Estrogens-deficient state in ovariectomized animals has been repeatedly shown to result in a rapid liver fat accumulation. [34,35,36] This is consistent with the current observation of a significant increase in the liver TAG in OVX group compared to the sham operated one. The exact pathogenesis of hepatic lipid accumulation seems to be very complex. As a whole, the general mechanism of liver fat accumulation involves an imbalance between lipid availability and lipid disposal. Hepatic steatosis can occur as a result of increased fat delivery into the liver (dietary fatty acids and plasma non-esterified fatty acids derived from adipose tissue), increased lipogenesis in liver, reduced fat oxidation, or reduced fat exportation in
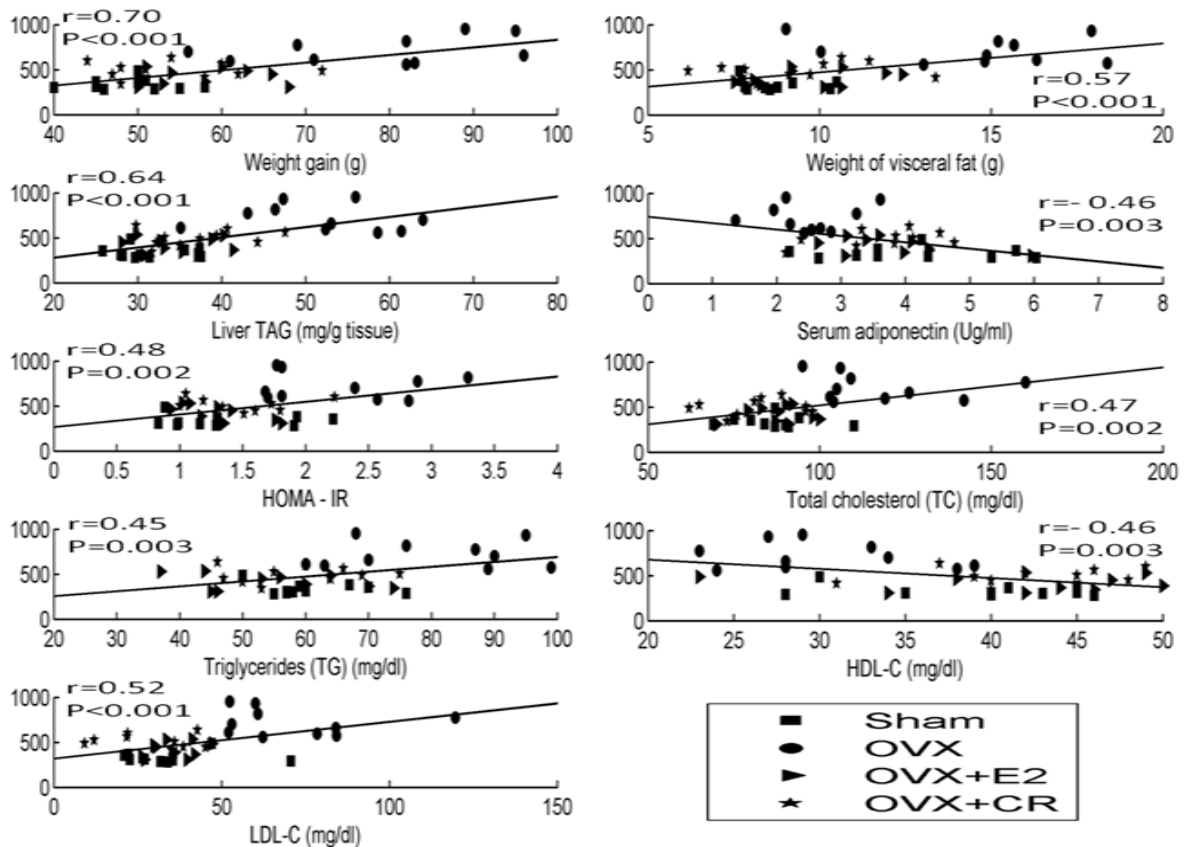
Fig. 4. Scatter diagram showing the significant positive correlations found between serum fetuin-A with weight gain, weight of visceral fat, liver triacylglycerol (TAG), homeostatic model assessment of insulin resistance index (HOMA-IR), total cholesterol (TC), triglycerides (TG) and low density lipoprotein- cholesterol (LDL-C); and significant negative correlations found between fetuin-A and high density lipoprotein- cholesterol (HDL-C) and serum adiponectin. r, Pearson's correlation coefficient for pooled data (n=40).

the form of triglyceride-rich lipoprotein secretion. [37] Visceral fat, via its unique location and enhanced lipolytic activity, releases toxic-free fatty acids, which are delivered in high concentrations directly to the liver; therefore, excess intra-abdominal fat deposition after estrogens withdrawal can primarily and partially explain hepatic fat accumulation. [38]

Our results revealed that both caloric restriction and E_2 replacement were equivalent in decreasing liver TAG in comparison with OVX group. In line, Rector et al. demonstrated that caloric restriction can prevent the development of NAFLD in hyperphagic rats. [39] Nevertheless, Meyer et al. observed that CR may be beneficial for reducing liver lipid and lowering triglycerides in overweight

subjects without known NAFLD. [40] In a recent review, it was concluded that at present, there are no specific or effective pharmacological treatments available for NAFLD in older women, and lifestyle modifications with weight loss and exercise are regarded as first line treatments. [41]

After menopause, risk of T2DM, atherosclerosis and coronary artery disease are more frequent, where insulin resistance and atherogenic lipid profile appear. [8, 42] Supporting these evidences, the current study confirmed development of insulin resistance thirteen weeks after ovariectomy. Additionally, we found a significant increase in the atherogenic lipid profile; TC, LDL-C and TG as well as a significant decline in the

protective HDL-C in the OVX group compared to the sham controls.

Interestingly, both E_2 replacement and CR succeeded to ameliorate these metabolic derangements compared to the OVX rats; decreased insulin resistance and almost normalized lipid profile. Yet, differences between E_2 and CR groups were insignificant. These findings are in accordance with previous researches demonstrated that caloric restriction can attenuate dyslipidaemia and/or improve insulin sensitivity in overweight people [40] and in streptozotocin induced diabetic rats. [43] Moreover, Stubbins et al. [44] proved that estrogen protected female mice from developing liver steatosis and insulin resistance.

Animal studies have consistently shown that fetuin-A knockout mice are resistant to weight gain and insulin resistance induced by high fat diet or aging. [17, 45] In addition, several human cross-sectional studies documented a positive association between high fetuin-A level and prevalent diabetes. [46, 47] The present study hypothesized that fetuin-A contributes, at least in part, to pathogenesis of menopausal insulin resistance and dyslipidaemia. Our findings revealed that fetuin-A is increased in OVX rats versus sham operated rats, while protective effects of E_2 administration and CR were associated with decreased serum fetuin-A. Moreover, fetuin-A was positively correlated with glycaemia, insulinaemia, insulin resistance as well as the atherogenic lipid profile. Although Fetuin-A, to our Knowledge, was not previously studied in OVX rat model, Ix et al. found that higher fetuin-A concentrations are strongly associated with metabolic syndrome and an atherogenic lipid profile in humans. [19] In agreement to our results, plasma fetuin-A levels

were positively correlated with fasting insulin and associated with higher risk of developing T2DM in USA women. [48]

In the present study, a significant positive correlation was found between the level of fetuin-A and that of liver TAG. Similarly, Stefan et al. found a significant correlation between plasma levels of fetuin-A and liver fat content in subjects with increased risk of the metabolic syndrome. [49] This highlights a possible increased fetuin-A secretion from the liver with increased TAG deposition after surgically induced menopause. When hepatic steatosis was corrected by E_2 or CR, fetuin-A level was significantly reduced. This is consistent with Choi et al. who demonstrated that CR significantly reduced expression and circulating levels of fetuin-A, alleviated hepatic steatosis as well as improved several cardiovascular risk factors including; body weight, central obesity, glucose, total cholesterol and triglyceride levels in obese rats and humans with T2DM. [50]

Fetuin-A was previously suggested to decrease the activity of insulin receptor tyrosine kinase, and thus inhibiting insulin receptor autophosphorylation. [16] This may provide a mechanism of insulin resistance offered by fetuin-A. However, further investigations are required to confirm or deny this mechanism in OVX rats.

Previous researches suggested a mechanism linking fetuin-A and adiponectin in the development of insulin resistance and dyslipidaemia. Therefore, we investigated serum adiponectin level and observed hypoadiponectinaemia in OVX rats that was reversed by E_2 therapy and CR. We also

demonstrated a significant negative correlation between fetuin-A and adiponectin levels, suggesting another mechanism of fetuin-A in the development of insulin resistance. In accordance to our results, Hennige and colleagues [18] demonstrated that fetuin-A suppressed adiponectin mRNA in cultured human adipocytes, and treatment of wild-type mice with fetuin-A lowered serum adiponectin levels. They concluded that the effect of fetuin-A on adiponectin is specific because fetuin-A treatment did not affect levels of mRNA encoding other adipocytokines such as leptin or resistin.

Adiponectin has been shown to have insulin-sensitizing effects including; stimulation of fatty acid oxidation and glucose uptake in skeletal muscle and suppression of glucose production in the liver. [51] Experimental studies suggested that administration of adiponectin ameliorates insulin resistance in T2DM mice. [52] For those reasons, adiponectin is considered to be one of the major insulin-sensitizing hormones strongly involved in insulin resistance-related disorders.

Collectively, these studies suggest that the liver-secreted protein, fetuin-A, inhibits generation of adiponectin in adipose tissue. Higher fetuin-A and lower adiponectin may contribute to obesity-induced insulin resistance. [18]

In addition to its insulin sensitizing effects, our finding of improved lipid profile in the E_2 and CR groups could be attributed to higher adiponectin concentrations compared to the OVX group. In fact, a growing body of literature data suggested that adiponectin has a direct effect on the regulation of lipid metabolism. Decreased adiponectin concentrations have been linked to higher LDL-C and TG concentrations probably

due to adiponectin directly affecting lipoprotein lipase. [53] Two large cross-sectional studies indicated that circulating adiponectin concentrations are negatively correlated with triglyceride concentrations and strongly positively correlated with plasma HDL-C concentrations. [54]

In summary, the probable mechanism of metabolic abnormality in obese menopausal female is with caloric excess, due to menopause induced hyperphagia, there is fatty acid excess fueling hepatic TAG synthesis and steatosis. Fatty liver may lead to greater serum fetuin-A concentrations. Higher fetuin-A levels may be responsible for suppression of adiponectin secretion. Both increased Fetuin-A and decreased adiponectin contribute, at least in part, to the increased insulin resistance and dyslipidaemia. So with E_2 replacement or with caloric restriction, all these processes are prevented or reversed; reduced TAG deposition in the liver, decreased Fetuin-A and increased Adiponectin with a final result of enhancement of insulin sensitivity and amelioration of dyslipidaemia.

Conclusion

From our work results, we can conclude that Fetuin-A could be one of the factors contributing to the development of menopausal metabolic complications related to menopausal obesity. Through modulation of fetuin-A and adiponectin levels, caloric restriction alone can alleviate weight gain, liver TAG accumulation, dyslipidaemia and insulin resistance associated with menopause without the hazardous effects of E_2 replacement.

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