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Fetuin A Levels Among Different Grades Of Obesity With Its Potential Link To Its Complication With Elaboration Of Physical Training Effects In Rats

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Keywords

- Obesity
- Fetuin-A
- Adiponectin
- AMPK
- Exercise

Abstract

We aimed to examine the link between fetuin A and different grades of obesity with its potential link to its complication and to determine the effect of exercise on its levels as well as on obesity complications. Methods: 40 male rats were classified based on adiposity index by using cluster analysis to 4 groups: G1: normal weight with no physical training n=10, G2: Overweight n=9, G3: Obese n=11 and G4: normal weight with physical training n=10. Albumin and creatinine were determined in urine and serum levels of fetuin-A, adiponectin, TNF-α, MDA, GSH, lipid profile and HOMA IR were measured. Also, liver NFkappa and renal relative AMPK mRNA expression were determined. Results: Fetuin–A, MDA, TNF-α, LDL, TG, HOMA IR, NFkappa, Adiposity index and ACR were significantly higher while adiponectin, GSH, HDL and relative AMPK mRNA expression were significantly lower in group2,3 as compared to group1,4 and as compared to each other. While, group3 showed significant increase in ACR, as compared to group1,2,4 but there was no significant change in ACR in group2. Group4 showed significant increase in adiponectin, GSH, HDL and relative AMPK mRNA expression and significant decrease in fetuin-A, TNF-α, MDA, HOMA IR, LDL ,TG, NFkappa, adiposity index and ACR as compared to group2,3. Also, positive correlation between fetuin-A and Adiposity index, ACR and NFkappa with negative correlation between it and adiponectin detected in group2,3,4.Conclusion: Fetuin-A level is directly proportional to obesity grades and its complication. Also, exercise appears to have protective role by decreasing fetuin-A level.

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INTRODUCTION

Obesity is related with an increased danger of early mortality and has reached epidemic proportions globally [1]**.** Obesity is considered an issue in all nations, not only those with a high standard of living, but it rapidly increases in low- and middle-income nations, most notably in urban areas. Obesity is not just a cosmetic consideration; it is a perilous situation that is directly destructive to one's health. Obese individuals are at a higher danger of coronary heart disease, stroke, hypertension, diabetes, and a variety of other chronic conditions [2].

Obesity is also a significant danger aspect for developing kidney disease; the potential nephrotoxic effect of obesity is caused by impairing the production of certain adipose tissue cytokines, such as adiponectin, leptin, and fetuin-A in addition to development of inflammation, oxidative stress, abnormal lipid metabolism, activation of the renin-angiotensin-aldosterone system, increased insulin production, and insulin resistance, as well as obesity has indirect nephrotoxic effects as it triggers the occurrence of diabetes and hypertension which are two major risk factors for development of chronic kidney disease (CKD) [3].

Obesity and being overweight are totally different from each other; weight gain could be linked to muscle, bone, fat, or body fluids. A person who is obese has an abnormal high and unhealthy percentage of body fat [1]**.**

Fetuin-A is a 64kDa glycoprotein that is abundant in human blood at quantities ranging from 300-1000 (μg/ml). Fetuin-A is mostly produced and released by the liver and adipose tissue in adult humans. Fetuin-A is an inhibitor of ectopic calcification on a systemic level, which is also associated with vital parameters of metabolic problems as insulin sensitivity, glucose tolerance, circulatory lipids, and pro- and anti-inflammatory proteins [4].

In hepatocytes and skeletal muscle, fetuin-A binds to and inhibits the insulin receptor tyrosine kinase. Additionally, fetuin-A stimulates the expression of cytokines and inhibits the generation of adiponectin [5]**.**

Brix et al., [6] assessed that Fetuin-A levels are increased in morbidly obese populations compared to controls and decrease after substantial weight reduction produced by bariatric surgery, indicating a positive relation between fetuin A and truncal obesity and dyslipidemia, particularly hypertriglyceridemia. Fetuin A may be an excellent predictor of visceral adiposity and dyslipidemia, particularly TG and TG-rich lipoproteins, in non-diabetic heart disease patients with a relatively lean body mass. Fetuin-A may have a role in the progression of non-alcoholic fatty liver disease and type 2 diabetes [7].

Obesity (especially that characterized by an increase in visceral fat) as a result of excess caloric intake together with a lack of physical exercise, results in higher FFA and proinflammatory cytokine levels in the circulation with reduction of adiponectin levels. FFA directly stimulates the liver's fetuin-A production [8]

Weight reduction produced by a healthy lifestyle decreases fetuin-A. Furthermore with exercise alone, circulating fetuin-A was founded to be decreased, and this change was related to reduction of insulin resistance [9].

Renal disease including CKD, nephrolithiasis and kidney cancers are among the more deceptive

properties of obesity, nevertheless have extensive damaging results, leading to significant increase illness and death and high costs for population and the entire society. Interventions to control obesity could have useful outcomes in stopping or delaying the progression of CKD. So, it is important to advance plans toward understanding of the relation between obesity and renal illness [3]

Materials and Methods:-

1-Animal care:

The present work was conducted on 40 male Albino rats of local strain aged (24-28 weeks) weighted (150-170g). The rats were kept in a clean animal cages, in a laboratory room which is prepared for animal housing animals had free access to food and water all the time and room temperature maintained at (22 -25) °c with a 12-hour light-dark cycle. All procedures were accepted by ethical committee of faculty of medicine by code no: (34964/10/21), Tanta University

2-Experimental protocol

After two weeks of acclimatization, by a random technique rats were divided into three primary groups.

Normal diet (ND) group (n=10 rats): received normal chow for 24 weeks [10]

Sedentary high-fat diet (HFD) (n=20 rats): received HFD for 24 week. They were kept in their cages during the duration of the experiment without any kind of exercise. [11].

Physical training plus high-fat diet group (n=10 rats): received HFD for 24 week. In the last 16 weeks they performed their exercise protocol [12]

3-Diet composition:

The ND composed of protein (20% casein), (15% corn oil), (55% corn starch), (5% salt combination), and (5% vitaminized starch). The HFD is consisted of (70% fat, 20% carbs, and 10% protein). It is composed of cooked cow fat, full cream milk, bread and green vegetables [13] both diets were obtained from El Gomhorria Pharmaceutical Co.

4-Exercise protocols (Swimming).

Before the start of the training, the rats were acclimatized to the water. By allowing them to swim in water (31 °C) for 30 minutes once a day for five days. Then, swimming protocol started in which the rats were trained by swimming for 60 minutes per day, five days /week for 16 weeks. The water tanks were 50 cm in height and 30 cm in diameter [12]

For all animals: body weight was followed up for all groups every four weeks

At the end of the experiment: urine was collected in a metabolic cage. Urinary albumin and Urinary creatinine concentration were measured and albumin-to-creatinine ratio in the urine (ACR) was calculated. Then, rats were anaesthized by 0.1 ml intraperitoneally of 1% sodium barbiturate. After anesthesia animals were decapitated and serum samples were collected in clean test tubes and centrifuged at 3000 rpm for 15 minutes before being transferred to a clean cuvette tube maintained at -20^{o}C. Through a mid-ventral abdominal incision, the testes were visualized and the attached fat pads were separated from surrounding tissue and bilaterally excised (epididymal fat) also, retroperitoneal fat pads located on the kidneys were excised and through a more rostral mid-ventral abdominal incision adipose tissue from the stomach (omental) as well as from multiple locations within the mesentery proper (mesenteric) were collected (visceral fat).

Finally all this fat pads were weighted. Liver and kidney were dissected for tissue homogenate.

Adiposity index

Total body fat was measured by finding the sum of epididymal fat, retroperitoneal fat and visceral fat. For calculation of adiposity index, the following equation was used (Total body fat/final body weight) × 100. The adiposity index was used as a measure of adiposity, because the degree of fat tends to increase gradually with obesity [11].

Cluster analysis of the degree of adiposity

Actually, there are no defined standard criteria for identifying overweight or obesity in laboratory animals (e.g., rats and mice). Cluster analysis based on the adiposity index of rats fed ND, HFD was used to construct comparable groups in terms of adiposity level and to differentiate degrees of adiposity in these rats [11].

Cluster analysis is a statistical technique for data categorization and reduction. This approach enables the classification of large volumes of undivided data into subgroups based on shared traits. The analysis provides a linkage tree that enables the assignment of cases to subsets, referred to as clusters. The linking approach may then be used to organize these clusters. A close connection distance indicates that the examples are comparable. A significant connection distance indicates that the situations are distinct. The nearest neighbor methodology (single linkage method) was utilized to cluster the data in this research, and the similarity coefficient was the median Euclidean distance. Finally, Dendrogram were used to characterize each cluster as shown in figure (1) [11].

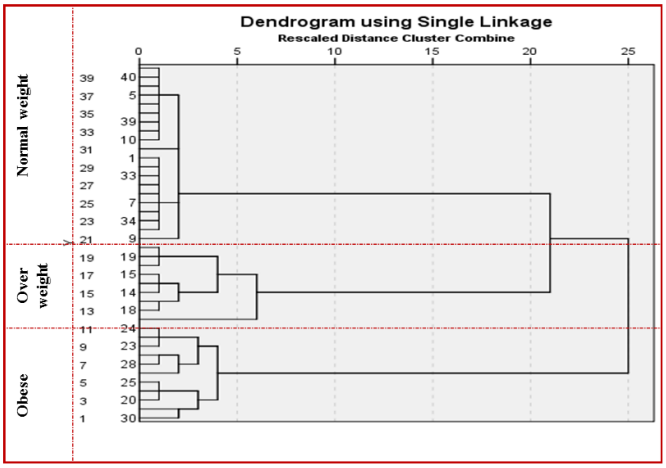

Figure 1: Dendrogram of cluster analysis according to the adiposity index of Normal diet group, Sedentary high-fat diet and Physical training plus high-fat diet group.

Determination of groups following cluster analysis

After cluster analysis, the animals were sub grouped depending on their degree of adiposity (normal weight, overweight and obese). So final classification of groups were: group1: (normal weight with no physical training n=10), group 2: Overweight (n=9), group 3: Obese (n=11) and group 4: normal weight with physical training (n=10)

After selecting the groups, using stored sera the following were measured:

Serum fetuin-A ELISA kit (Shanghai Sunred Biological Technology Co. Ltd, China. Catalog no

201-11-0581). **Rat Adioponectin** ELISA kit (Shanghai Sunred Biological Technology Co., Ltd, China Catalog no 201-11-0759.**Rat (TNF-α)** ELISA kit (Shanghai Sunred Biological Technology Co. Ltd, China. Catalog no 201-11-0765). **Insulin** ELISA kit (Calbiotech Inc., 10461 Austin Dr, Spring Valley, CA, USA). **Serum reduced glutathione (GSH)** Biodiagnostic KitNo. GR2511(BiodiagnosticCo., Egypt). **Serum Malondialdehyde (MDA)** Biodiagnostic Kit No MD 25 29. **Serum fasting glucose level** (Egyptian company for biotechnology, Cairo, Egypt. Catalog number 250 001). **Serum HDL** (Biosystems S.A. Costa Brava, Barcelona. Spain. Cod no 11648), **Serum LDL** (Biosystems S.A. Costa Brava, Barcelona. Spain. Cod no 11579) and **Serum triglycerides** (Egyptian company for biotechnology, Cairo, Egypt. Cod no 314 009). **HOMA IR** was calculated by method described by [14].

Tissue homogenate:

Ice-cold saline was used to wash the liver and kidney three times then blotted on filter paper and homogenized in 50 mM potassium phosphate (pH 7.4). Centrifuged the homogenate in 7000×g for 10 min at 4°C and supernatant were stored at -80ºC and used for measurement of:

Liver tissue NFkappa ELISA kit (MyBioSource, San Diego, CA 92195-3308. USA. Catalog no MBS453975).

Detection of AMPK gene expressions in kidney tissues by quantitative real time PCR (qRT-PCR): Total RNA was derived from the renal tissue homogenate using Gene JET RNA Purification Kit (Thermo Scientific, # K0731, USA). according to the manufacturer's instructions. First-strand cDNA was synthesized from 5 µg of total RNA using Revert Aid H Minus Reverse Transcriptase (Thermo Scientific, #EP0451, USA). PCR reactions were performed using Power SYBR Green PCR Master Mix (Life Technologies). The primers sequences were as follow**: Rat AMPK** forward primer (5′-TCTCGGGGTGGTTCGGTG- 3′) and reverse primer (5′-GGGGACAGGATTTTCGGATT-3′) (GenBank Accession No. NM_023991.1) **rat β-actin** forward primer (5′-CGTTGACATCCGTAAAGACCTC-3′) and reverse primer (5′-TAGGAGCCAGGGCAGTAATCT-3′) (GenBank Accession No. NM_031144.3). The cycling pattern was as follows: one cycle at 95°C for ten minutes, followed by forty cycles of amplification at 95°C for fifteen seconds, 60°C for one minute, and 72°C for one minute. The cycle threshold (Ct) values for target genes and the housekeeping gene were established, and the relative gene expression was estimated using the $2^{-\Delta\Delta Ct}$ technique. [15]

Statistical analysis:-

Results were expressed as Mean ± SD and all statistical comparisons were done using the one-way ANOVA test, followed by Tukey's post hoc analysis, with p values less than 0.05 indicate statistical significance. The analysis was conducted using the statistical package for social science software (SPSS version 22.0). The Pearson correlation coefficient (Pearson r test) was used to determine the strength and relationship of two variables. r = (-1 to +1).

1. -1 means there is a strong negative correlation
2. +1 means that there is a strong positive correlation
3. 0 means that there is no correlation (this is also called zero order correlation).

Results:

The result of this work revealed that in overweight and obese group there was significant increase (P≤0.05) in: serum Fetuin –A, liver tissue NFkappa, Adiposity index (as shown in table 1), LDL, TG, HOMA IR (as shown in table 2) ,serum MDA and TNF-α (as shown in table 3). However, there was significant lowering (P≤0.05) in adiponectin, renal relative AMPK mRNA expression (as shown in table 1), HDL (as shown in table 2) and GSH (as shown in table 3) as compared to control, swimming groups and as compared to each other.

In addition to the above factors contributing to obesity, obese group showed significant increase (P≤0.05) in ACR (as shown in table 1), as compared to control, overweight, swimming groups while there was insignificant change(P≥0.05) in ACR in overweight group (as shown in table 1).

There was positive correlation between serum fetuin-A and adiposity index, ACR and NFkappa and there was a negative correlation between it and adiponectin in overweight group (as shown in figure 2)

Also, there was positive correlation between serum fetuin-A and adiposity index, ACR and NFkappa and there was a negative correlation between it and adiponectin in obese group (as shown in figure 3)

On other hand, swimming group presented significant increase (P≤0.05) in: serum adiponectin and renal relative AMPK mRNA expression (as shown in table 1), serum HDL (as shown in table 2) and GSH (as shown in table 3). On the other hand there was significant decrease in serum fetuin-A , liver tissue NFkappa , adiposity index and ACR (as shown in table 1) serum LDL, TG, and HOMA IR (as shown in table 2) TNF-α, MDA (as shown in table 3) as compared to both overweight and obese group.

Also, there was positive correlation between serum fetuin-A and adiposity index, ACR and NFkappa and there was a negative correlation between it and adiponectin in swimming group (as shown in figure 4).

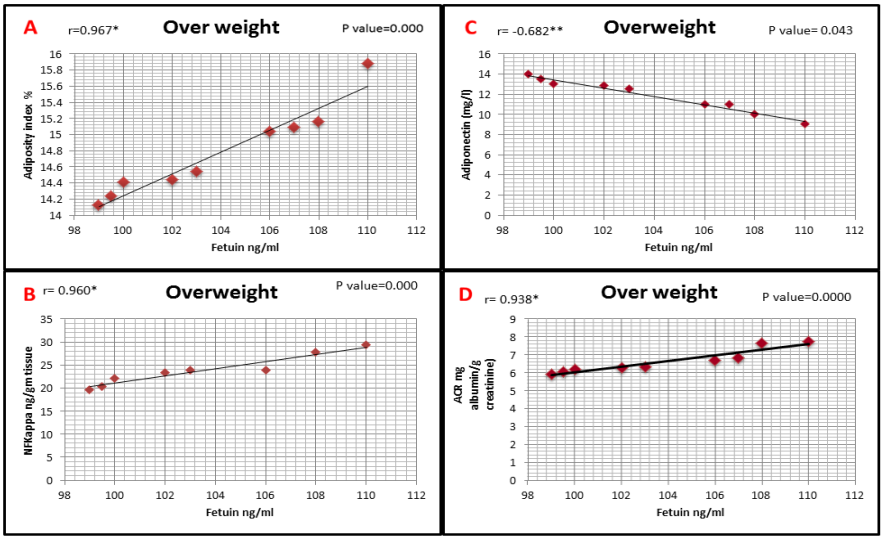

Figure 2:-Correlation of serum fetuin-A with (A)Adiposity index (B) liver tissue NFkappa (C) Serum adiponectin (D) Urinary ACR in overweight group.*denotes statistical significance at P≤0.05 (positive correlation).**denotes statistical significance at P≤0.05 (negative correlation)

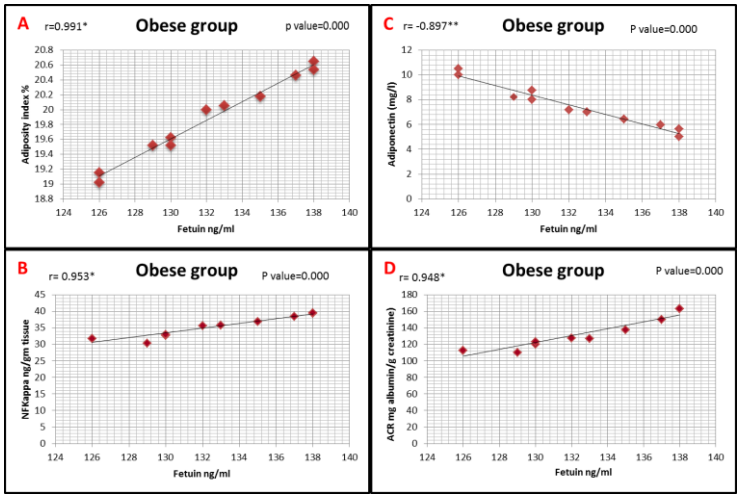

Figure 3:-Correlation of serum fetuin-A with (A)Adiposity index (B) liver tissue NFkappa (C) Serum adiponectin (D) Urinary ACR in Obese group.*denotes statistical significance at P≤0.05 (positive correlation).**denotes statistical significance at P≤0.05 (negative correlation).

Table 1:- Adiposity index, Serum fetuin, liver tissue, NFkappa, Renal tissue relative AMPK, Serum Adiponectin and urinary ACR among studied groups (Mean value ±SD)

Groups / Parameters	Normal weight without physical training (n=10)	Sedentary high-fat diet		Normal weight with Physical training (n=10)	F value	P value
		Overweight (n=9)	Obese (n=11)			
Adiposity index (%)	11.14±0.41	14.77±0.57 ^{acd}	19.88±0.56 ^{abd}	11.27±0.31^{bc}	802.54	0.000 (P≤0.05)
Fetuin ng/ml	87.88±2.74	103.83±4.04 ^{acd}	132.18±4.42^{abd}	90.47±2.74^{bc}	341.46	0.000 (P≤0.05)
NFkappa ng/gm tissue	12.12±2.02	24.20±3.25^{acd}	35.05±3.29^{abd}	12.73±2.42^{bc}	158.42	0.000 (P≤0.05)
Relative renal AMPK mRNA expression	1.00±.049	0.58±0.057 ^{acd}	0.19±0.039^{abd}	0.96±0.042^{bc}	673.11	0.000 (P≤0.05)
Adiponectin (mg/l)	23.88±2.70	14.87±2.22^{acd}	11.66±1.12 ^{abd}	23.60±2.76^{bc}	89.19	0.000 (P≤0.05)
ACR (mg albumin/g creatinine)	5.32±0.54	6.67±0.67^{c}	131.54±19.55^{abd}	6.51±.68^{c}	392.36	0.000 (P≤0.05)

1. [a] P≤0.05 versus control group
2. [b] P≤0.05 versus over weight
3. [c] P≤0.05 versus obese group
4. [d] P≤0.05 versus swimming

Table 2:- Serum HDL, LDL, TG and HOMA IR among studied groups (Mean values ±SD)

Groups / Parameters	Normal weight without physical training (n=10)	Sedentary high-fat diet		Normal weight with Physical training (n=10)	F value	P value
		Overweight (n=9)	Obese (n=11)			
HDL (mg/dl)	50.34±2.33	31.87±2.79 ^{acd}	23.55 ±2.35 ^{abd}	50.37±1.49 ^{bc}	364.65	0.000 (P≤0.05)
LDL (mg/dl)	89.53±3.08	120.52±1.31 ^{acd}	127.82±2.32^{abd}	91.22±1.49 ^{bc}	830.22	0.000 (P≤0.05)
TG (mg/dl)	145.55±7.99	168.60±1.92 ^{acd}	181.77±3.46^{abd}	146.90±4.29 ^{bc}	129.94	0.000 (P≤0.05)
HOMA IR	2.43±0.52	5.99±0.54 ^{acd}	9.13±0.89^{abd}	2.68±0.49 ^{bc}	253.35	0.000 (P≤0.05)

1. [a] P≤0.05 versus control group
2. [b] P≤0.05 versus over weight
3. [c] P≤0.05 versus obese group
4. [d] P≤0.05 versus swimming

Table 3:- Serum TNF α, Serum MDA and Serum GSH among studied groups (Mean value ±SD)

Groups	Normal weight without physical training (n=10)	Sedentary high-fat diet		Normal weight with Physical training (n=10)	F value	P value
Parameters		Overweight (n=9)	Obese (n=11)			
serum TNF α (ng/l)	2.79±0.50	18.42±0.86^{acd}	20.31±1.22^{abd}	2.82±0.28^{bc}	1401.37	0.000 (P≤0.05)
Serum Malondialdehyde (mg/dl)	1.34±0.16	2.58±0.33^{acd}	3.50±0.49^{abd}	1.60±0.26^{bc}	88.28	0.000 (P≤0.05)
Serum reduced glutathione (mg/dl)	3.01±0.11	1.46±0.22^{acd}	0.95±0.07^{abd}	2.78±0.38^{bc}	196.69	0.000 (P≤0.05)

1. [a] P≤0.05 versus control group
2. [b] P≤0.05 versus over weight
3. [c] P≤0.05 versus obese group
4. [d] P≤0.05 versus swimming

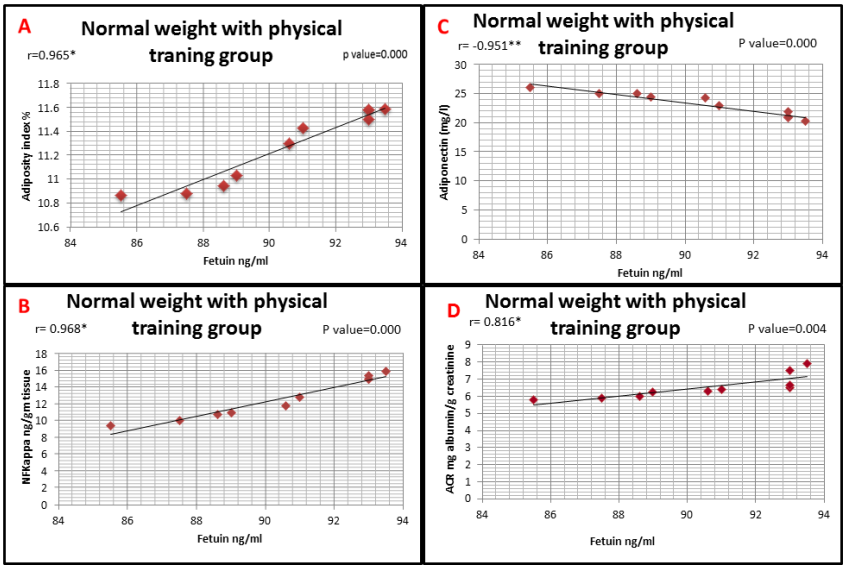

Figure 4:-Correlation of serum fetuin-A with (A) Adiposity index (B) liver tissue NFkappa (C) Serum adiponectin (D) Urinary ACR in normal weight and physical training group.*denotes statistical significance at P≤0.05 (positive correlation).**denotes statistical significance at P≤0.05 (negative correlation).

Discussion

Changes in lifestyle and food have resulted in a rise in the occurrence of overweight and obesity during the last several decades. Obesity has emerged as a critical public health issue, posing a serious danger to human health around the globe [16]

The diet used in this work was sufficient to stimulate obesity in rats. The intake of high caloric diet induced significant differences in the adiposity index between the HFD and ND groups and difference in adiposity index within HFD group.

The HFD induced changes that look like those of human comorbidities caused by obesity, such as hypertriglyceridemia, insulin resistance and microalbuminuria [17].

In this research obesity was classified into different grades using cluster analysis depending on the adiposity index. Pervious study has used cluster analysis to classify adiposity in an experimental induced obesity in animal like to

those defined for human obesity (i.e., overweight and obese) [18]

This method allowed us to determine Fetuin A Levels among different grades of obesity with its potential link to obesity complication with elaboration of effect of physical training.

The significant elevation of TG and LDL with reduction of HDL level in overweight and obese group may be due to the resistance to the action of insulin on lipoprotein lipase at the peripheral tissues [19]. This was confirmed in the present work by elevation of HOMA IR in obese and overweight groups.

The present work showed positive correlation between serum Fetuin A concentration and adiposity index in both overweight and obese group.

Adipocyte dysfunction could be the cause of elevated Fetuin A levels in both groups [20].

This is in agreement with Ismail et al., [21] who found that Fetuin-A knockout mice did not gain weight on a HFD, this suggest that high Fetuin-A levels may lead to obesity.

Also, formation of Alpha 2-HS Glycoprotein is enhanced by FFAs via the NFkappa (which combined to Fetuin-A promoter that in turn up-regulates Fetuin-A gene expression). So, fatty acids could efficiently stimulate Fetuin-A synthesis and secretion from adipocytes and hepatocytes [22]. This is in agreement with the present study as there was positive correlation between serum Fetuin-A level and liver NFkappa in HFD groups.

In addition, the role of Fetuin -A in the development and progression of obesity- related complication was explained by Trepanowski et al., [23] who reported that Fetuin-A impairs insulin receptor signaling and activation of TLR4 (member of the toll-like receptor family), which is responsible for adipocytes dysfunction, hepatocyte triacylglycerol accumulation and liver inflammation and fibrosis.

The significant lowering of adiponectin level in present study agrees with Di Chiara et al., [24] who reported that accumulation of visceral fat is associated with hypoadiponectinemia.

Trepanowski et al., [23] stated that in adipocytes as well as monocytes, Fetuin-A administration increases the expression of pro-inflammatory cytokine mRNA while decreasing the expression of adiponectin mRNA. As a result, Fetuin-A acts as an independent predictor of circulating adiponectin [25].

This was proved by negative correlation between serum Fetuin A and adiponectin among group2 and 3.

As regard to inflammatory status observed in this study that was in the form of elevation of TNFα, MDA and lower of GSH, it can be explained by activation of adipocytes COX-2 and PGE2 /EP3 signaling during adipocytes hypertrophy that contribute not only to increase proinflammatory adipokines but also decrease in adiponectin production mainly via activation of NFkappa mediated inflammatory pathway [26].

NFkappa is transcriptional factor that inter to the nucleus and binds to DNA and up-regulates the transcription of many inflammatory genes as (TNF α) also, NFkappa induce a major oxidative stress signaling pathway in tissues [27].

Lipid peroxidation in the form of MDA elevation in obesity could be due to cumulative and progressive cell injury caused by the pressure exerted by an increased body mass . So, injured

cells release cytokines as TNF-α which generates ROS from the tissues which in turn induce lipid peroxidation [28].

Also, the increase in the free fatty acids levels by hypertriglyceridemia cause increase in lipid peroxidation and elevation of MDA and this leads to alteration in the oxidant-antioxidant balance [29].

On the other hand, ACR was significantly higher in obese group only, which indicate renal impairment. This is in agreement with Li et al., [10] who stated that CKD is evidenced by the presence of proteinuria or a decreased glomerular filtration rate.

Microalbuminuria is defined as urinary albumin excretion of 30 to 300 mg/24 hours if measured in a 24-hour urine collection, or as 30 to 300 mg albumin/g creatinine when measured using the ACR in spot urine collection [30]

The imbalance between lipogenesis and lipolysis in the kidney tissue which result in renal lipid peroxidation may be the cause of elevated ACR in this group [31].

In addition, there are many other pathways by which obesity might contribute to renal disease. The mechanisms leading to both may be interrelated through crosstalk between fat, kidney and liver via Fetuin-A [32]

Present results suggest that: Fetuin-A play a role as a co-factor in development of renal impairment associated with different grades of obesity as proved by positive correlation between serum Fetuin A and ACR in HFD groups.

Other factors which are affected by obesity and may contribute to renal impairment through their relation to Fetuin-A, are: decrease adipokine as adiponectin, increase proinflammatory cytokines as TNF-α, insulin resistance, dyslipidemia and imbalance between free radical production and antioxidant defenses as observed in the present work by positive correlation between serum Fetuin-A and adiposity index and ACR and negative correlation with adiponectin.

On other hand renal impairment in obese group could be due to reduction of adiponectin that reduces renal relative AMPK expression. As it was observed that adiponectin knockout mice have high level of microalbuminuria, oxidative stress and podocyte damage which was reduced after exogenous adiponectin administration. This effect is mediated through adiponectin effect on the AMPK pathway in podocytes [33].

Also, in the present study, we recognized that renal relative AMPK expression was reduced together with reduction in adiponectin level in HFD groups and this is in agreement with Declèves et al., [34]

Sharma et al, [35] also reported that glomerular AMPK was induced by adiponectin and AMPK activity was reduced in the glomeruli in adiponectin knockout mice.

On the other hand the adoption of a healthy lifestyle, including physical exercise, is an important non-pharmacologic approach for preventing obesity and its complications.

From this perspective, the effect of exercise intervention for 16 week was investigated in this study.

Interestingly, physically trained group showed that serum Fetuin-A levels were significantly decreased. In addition there was significant decrease in liver tissue NFkappa and ACR. Also, there was significant increase in serum adiponectin and renal relative AMPK expression.

Notably, the beneficial effect of exercise was mainly reflected in significant decreases of HOMA IR, adiposity index, LDL and TG and significant increase in HDL. Also we demonstrated that swimming prevented intense oxidative stress and enhanced antioxidant status in comparison with HFD groups, as evidenced by reductions in MDA, and NFkappa, in addition to elevation of GSH.

This finding can be explained by increased lipolysis during exercise due to increased lipoprotein lipase activity that improves lipid profile and HOMA IR. This is in agreement with the findings of Ragi et al., [36].

Also, Exercise enhances synthesis of mitochondria, accelerates glucose transportation and lipid decomposition also increases phosphorylation of AMPK that improves glucose intake and energy metabolism in the body [37]

Improvement in ROS markers was also reported by Karabulut et al., [38] who described that exercise intensify antioxidant defense system by increasing superoxide dismutase enzyme expression and activity

Also decrease in visceral adiposity induced by exercise cause decrease in TNFα and this suggest that training could be necessary for intervention strategy for both the inhibition and treatment of the inflammatory state of obesity and its related complications [39]. This could explain the decrease in ACR in physically trained group.

Improvement in adiponectin and renal relative AMPK expression in physically trained group resulted from increase in the expression of adiponectin 5' AMP-activated protein kinase. Moreover adiponectin through AMPK pathway activation could increase fatty acid oxidation and glucose uptake [40].

There was positive correlation between Fetuin- A level and adiposity index in physically trained group. This is in agreement with Kavalakatt et al., [41] who reported that exercise reduce Fetuin-A in obesity.

Also, long-term exercise decrease Fetuin-A and FFAs which resulted in less TLR4 signaling leading to improvement of insulin sensitivity. Also it was reported that after exercise there was a significant correlation between Fetuin-A and insulin resistance in liver, but not in skeletal muscle, so the exercise-induced decrease of Fetuin-A is mainly linked to hepatic glucose production, regardless the changes in systemic inflammation [42]

Also, Exercise modulates activation of the NFkappa signaling cascade that inhibits TNF-α gene expression and increase expression of genes encoding mitochondrial SOD, which keep cellular oxidant/antioxidant homeostasis during exercise [43]. SO, exercise counteract obesity and its complication by reducing FFA with subsequent decrease of serum Fetuin-A and this interrupt pathways that are responsible for crosstalk between fat, liver and kidney in obesity. This was proved by positive correlation between serum Fetuin-A and adiposity index and ACR and negative correlation between serum Fetuin-A and adiponectin in physically trained group.

Conclusions

We conclude that Fetuin-A concentration is directly proportional to grades of obesity as well as to obesity-related complication. Also it is clear that Fetuin-A is a link between grades of obesity and

its complication through its effect on adiponectin that is the cross talk pathways between liver, kidney and adipose tissue. Also Exercise reduces Fetuin-A levels and this increase the scope how reduction of Fetuin-A prevents obesity and its complication .So, further studies are recommended to explore drugs that counter act Fetuin-A either at level of production or at site of action

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