

*Full Length Research Paper*

# Moderate exercise training has anorexogenic effect associated with improved oxidative stress in obese women

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Endocrine derangement and oxidative stress are two distinguishing features of obesity that have limited the success rate of various management strategies, especially physical activities. The objective of the current study was to examine the effect of 8 weeks of moderate-intensity regular exercise training on oxidative stress, appetite and weight loss in obese women compared with normal-weight women. Sixteen normal-weight (body mass index (BMI) < 25 kg/m<sup>2</sup>) and fifteen obese women (BMI > 30 kg/m<sup>2</sup>) exercised on the bicycle ergometer at moderate intensity for 30 min, 3 times per week for 8 weeks. Blood samples were collected at the first day of training and 72 h after the completion of the training program which were then used for the measurement of F2-Isoprostanes, glutathione (GSH), oxidized glutathione (GSSG), leptin, adiponectin and nesfatin-1. Eight (8) weeks of training resulted in lower BMI, insulin, GSSG (P < 0.05), leptin (P < 0.01), and F2-Isoprostanes (P < 0.001) and higher nesfatin-1 levels (P < 0.01) relative to the levels at the pre-test stage in obese women. Also  $\Delta$ leptin levels after exercise were positively correlated with  $\Delta$ F2-Isoprostanes and  $\Delta$ BMI. Eight weeks of moderate-intensity regular exercise program did not only induce weight loss and improve oxidative stress, but also modified insulin, leptin and nesfatin-1 concentrations, particularly in obese women.

**Key words:** Endocrine derangement, oxidative stress, obesity, exercise, weight loss, leptin, nesfatin-1.

## INTRODUCTION

Obesity is associated with serious metabolic complications which are anticipated to be the major causes of mortality in modern history (Olshansky et al., 2005). Vigorous efforts have been made to counteract adiposity but unfortunately, have so far been met with disappointment. The realization that adipose tissue acts as an endocrine gland affecting whole-body energy

homeostasis was a major breakthrough toward a better molecular understanding of adiposity origin and its management possibilities (Kershaw and Flier, 2004). Leptin, adiponectin and nesfatin-1 are novel adipokines secreted by adipocytes with Leptin, and adiponectin are denominated energy-regulating hormones owing to their role in regulating overall energy balance and body fat

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over prolonged time while nesfatin-1 is considered as an appetite-regulating hormone (Friedman and Halaas, 1998; Mujumdar et al., 2011; Ramanjaneya et al., 2010).

Leptin is secreted in direct proportion to the nutritional condition and was proven to suppress energy intake as a response of adequate energy stores (Friedman and Halaas, 1998). Earlier data reported increased synthesis of leptin mRNA and serum leptin level in obese individuals that, when compared with non-obese individuals, brings into a hypothesis of leptin resistance (Emilsson et al., 1999). Adiponectin is a protein secreted mainly by differentiated adipocytes (Maeda et al., 1996) that has been reported to increase thermogenesis, weight loss, reduce serum glucose and lipid levels and to generate a negative energy balance by increasing energy expenditure (Spranger et al., 2006). Unfortunately, low adiponectin levels were recorded in obese individuals (Weiss et al., 2003). On the other hand, nesfatin-1 is a recently discovered potent anorexigenic peptide that induces satiety and strongly inhibits food and water intake, thereby reducing body weight (Shimizu et al., 2009). Nesfatin-1 was found to be a novel depot specific adipokine preferentially produced by subcutaneous tissue, with obesity- and food deprivation-regulated expression (Ramanjaneya et al., 2010). The aforementioned obesity associated dysregulation in adipokines level was shown to be a potent stimulator for the production of reactive oxygen by macrophages and monocytes; therefore, responsible for increased oxidative stress. In turn, oxidative stress is associated with an irregular production of adipokines, which contributes to further derangement in energy homeostasis and more adiposity (Esposito et al., 2006).

In this sense, glutathione and F2- Isoprostanes (F2-IsoPs) were studied as sensitive markers of oxidative stress. Glutathione is a tripeptide thiol that exists in two different forms; oxidized glutathione (GSSG) which is a marker of oxidative stress in cytosol and reduced glutathione ( $\gamma$ -glutamylcysteinyl-glycine; GSH) which represents the major non-enzymatic endogenous antioxidant defense system (Pompella et al., 2003). GSH also acts as a cofactor for glutathione peroxidases enzyme which is involved in protecting cells against reactive oxygen species (ROS) (Becker et al., 2003). F2-IsoPs are markers of lipid peroxidation that are generated when free radicals attack cell membranes catalyzing the peroxidation of esterified arachidonic acid (an omega-6 fatty acid found in the phospholipids of cell membranes) which are then cleaved and released into the circulation by phospholipases (Roberts and Morrow, 2000). Available data indicate that quantification of F2-IsoPs in plasma gives a highly precise and accurate index of oxidative stress (Morrow, 2005). Not surprisingly, the mechanisms behind the regulation of appetite or food intake and energy expenditure in obese individuals are extremely complicated which makes body weight regulation remaining an obstacle. Restricting energy

intake is generally unsuccessful and more than 90% of obese individuals regain lost body fat within 2 years (Vogels et al., 2005). An alternative to restricting energy intake is increasing physical activity which is considered the most important modifiable behavior to regulate body weight and to prevent and/or reduce obesity through increasing energy expenditure. However, it is controversial whether the net effect of exercise is to reduce leptin level extensively which stimulate appetite and raise energy intake (Black et al., 2005) or to reduce leptin level moderately which mitigate leptin resistance and suppress the appetite (Martins et al., 2013).

Moreover different exercise protocols may induce varying levels of ROS production, or antioxidant defenses according to intensity and duration of the exercise which could further alter the levels of adipokines and modulate the energy balance (Goto et al., 2003). Therefore the present study aims to examine the oxidative stress markers and energy -regulating hormones in obese women compared with normal-weight ones and to follow the effect of 8 weeks of moderate intensity regular exercise training on these stress markers and homeostatic hormones in order to understand the impact of exercise on appetite and weight loss.

## METHODOLOGY

### Subjects

Forty-five normal-weight and obese females participated in an exercise program, plasma samples for the assessment of biomarkers were obtained from thirty-one participants who completed the entire program. All the participants had no experience of formal physical activity and the study was conducted during the period from November, 2013 to January, 2014 in the Faculty of Physical Therapy outpatient clinic at Cairo University, Egypt during which they had *ad libitum* and received appetite questionnaires to fill. Informed consent was obtained from each patient and the study protocols conformed to the ethics guidelines of the Institutional Review Board. Participants filled out a questionnaire that included questions regarding personal characteristics, health, smoking and physical training history. Heights and weights were measured using a digital scale and a tape, and their body mass index (BMI) values were then calculated (weight in kilograms divided by the squared height in meters). Participants (all are women) were divided according to their BMI into two groups: 16 normal-weight subjects (body mass index (BMI) < 25 kg/m<sup>2</sup>) and 15 obese subjects (BMI >30 kg/m<sup>2</sup>). The exclusion criteria were smokers, vegetarian subjects, subjects who suffer from any cardiovascular, pulmonary, neurological or musculoskeletal abnormalities and subjects consuming antioxidants, exogenous anabolic-androgenic steroids, drugs and medication or dietary supplements that could affect their redox potential or physical performance.

### Physiological measurements

The percentage of body fat was determined by measuring the amount of subcutaneous fat in the triceps, suprailiac and thigh, then using these values as input for the Jackson and Pollock equation (Williams, 2002). V.O<sub>2</sub> max was calculated to measure how

efficiently the cells use oxygen for energy. Fitness level was calculated according to the following equation (Williams, 2002):

$$V.O_2 \text{ max (ml kg}^{-1} \text{ min}^{-1}) = 88.02 - 0.1656 \times [\text{weight (kg)}] + 2.76 \times [\text{time (min)}] + 3.716 \times (0)$$

### Experimental design

First, the maximum heart rate was determined for each person using the following formula:  $208 - (0.7 \times \text{age})$  (Tanaka et al., 2001). Then 31 subjects exercised on a bicycle ergometer at intensity 70% of each subject's maximum heart rate for 30 min, 3 times/week for 8 weeks. Exercise intensity was controlled using a belt heart rate sensor. Blood samples were collected from each participant pre-exercise or baseline: on the first day of training and post-exercise or at recovery period: 72 h after the completion of the training program (at the end of the 24th session). Participants were also told to fast for 12 h in both time points. Answered appetite questionnaires were collected by the end of the training period.

### Blood collection and analysis

At each time point, blood samples were collected from the antecubital vein into 10 ml vacutainer tubes (Vacutainer, Becton Dickinson, USA) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) solution and a preservative to reduce auto-oxidation. Blood was centrifuged at 1,200 rpm for 10 min at 4°C to obtain plasma. Plasma (100 µl) was divided into two portions, one of which was designated as glutathione which was deproteinized with equal volume of metaphosphoric acid reagent (5 g in 50 ml water) (Sigma-Aldrich 239275) and mixed vigorously. The mixture was allowed to stand at room temperature for 5 min then centrifuged at > 2,000 for 2 min. The supernatant has been carefully collected. Then the two plasma portions were stored at -80°C until time of analysis. Upon use, plasma was diluted (1:100) with 0.1 mM phosphate buffer, pH 7.4, and used for the measurement of the following parameters: FBG, TAG, Total cholesterol (TC), HDL-C, LDL-C, HbA1c%, F2-Isoprostanes, reduced glutathione (GSH), oxidized glutathione (GSSG), insulin, leptin, adiponectin and nesfatin-1.

### Biochemical analyses

#### GSH and GSSG determination

The total glutathione and oxidized glutathione were measured by an in-house modified method (Griffith, 1980; Kullisaar et al., 2003). For measurement of the glutathione content, the sample should be deproteinized by TEAM (triethanolamine) reagent. 4 M solution of TEAM reagent (Sigma-Aldrich T58300) was prepared by mixing 531 µl of triethanolamine with 469 µl of water. Then 0.005 µl of TEAM reagent was added to 0.1 ml in the glutathione designated tube which was then divided into two portions. The first portion was used for determining oxidized glutathione and the second portion for total glutathione.

#### Sample preparation for exclusive measurement of GSSG

2-vinylpyridine was used to derivatize reduced glutathione. 1 M solution of 2-vinylpyridine (Sigma-Aldrich, 13229-2) was prepared in ethanol by mixing 108 µl of 2-vinylpyridine and 892 µl of ethanol. Then 5, 10 µl of 2-vinylpyridine were added to the standard and sample, mixed well on a vortex mixer and incubated at room

temperature for 60 min.

#### Quantification of GSSG and total glutathione

The total glutathione and the oxidized glutathione were measured by an in-house modified method (Griffith, 1980) using a GSH assay kit (Cayman Chemical Company, Michigan, USA) in which total glutathione was analyzed through the oxidation of GSH by the sulfhydryl reagent 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5'-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. The glutathione disulfide (GSSG) formed can be recycled to GSH by glutathione reductase in the presence of NADPH. Glutathione content was calculated on the basis of a standard curve generated with a known concentration of glutathione. The amount of GSH was calculated as the difference between total glutathione and GSSG.

#### FBG, TAG, TC, HDL-C, LDL-C and HbA1c (%)

Fasting blood glucose (FBG), triacylglycerol (TAG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured using Dimension RxL Max Integrated Chemistry System (DADE BEHRING instruments Inc, USA) automated biochemistry analyzer. The %HbA1c was measured in whole blood with the Dimension system that automatically calculates concentrations of total hemoglobin (Hb) and HbA1c, the instrument then calculates % HbA1c as follows:

$$\% \text{HbA1c} = \frac{(\text{g/dl HbA1c})}{(\text{g/dl Hb})} \times 100$$

#### F2-Isoprostanes, leptin, adiponectin and nesfatin-1

The current study investigated the free F2-Isoprostanes in plasma which are initially formed *in situ* esterified in phospholipids, then released in free form by phospholipase action. Plasma level of free F2-Isoprostanes was measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cusabio Biotech, Hubei, China) according to the manufacturer's instructions. Each sample was examined in duplicate and the average value (mean) was used for data analysis. A commercial ELISA kit for human adiponectin, leptin and nesfatin-1 (RayBio®, Norcross, GA) was used and the assay was conducted according to the manufacturer's instructions. The concentrations of plasma insulin were determined by ELISA (kits provided by UBI MAGIWEL) then the homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated from fasting insulin and glucose by the following equation:

$$\text{HOMA-IR} = \frac{\text{fasting insulin (in microunits per milliliter)} \times \text{fasting glucose (in milligrams per deciliter)}}{405}$$

#### Statistical analysis

Results are expressed as mean ± standard deviation (SD). All statistical analyses were performed using Windows-based statistical package for social sciences (SPSS) (SPSS version 17.0; SPSS, Chicago, IL). Kolmogorov-Smirnov test was done to evaluate the distribution of variables. Data were log transformed if they did not adhere to normal distribution. Differences between means were analyzed by the Student's t-test, and post hoc Bonferroni was

**Table 1.** Physical and clinical characteristics of the study populations at baseline and after exercise.

Factor	Normal-weight (16)		Obese (15)		P1	P2	P3	P4
	Before exercise	After exercise	Before exercise	After exercise				
Gender (F/M)	16/0	16/0	15/0	15/0	-	-	-	-
Age (y)	32.82 ± 3.05	32.82 ± 3.05	32.24 ± 2.68	32.24 ± 2.68	P1 > 0.05	P2 > 0.05	P3 > 0.05	P4 > 0.05
Height (m <sup>2</sup> )	1.624 ± 2.9	1.624 ± 2.9	1.62 ± 3.41	1.62 ± 3.41	P1 > 0.05	P2 > 0.05	P3 > 0.05	P4 > 0.05
Body weight (kg)	62.24 ± 3.61	62.16 ± 3.68	91.4 ± 8.21	87.88 ± 7.56	P1 < 0.001	P2 > 0.05	P3 > 0.05	P4 < 0.001
BMI (kg/m <sup>2</sup> )	23.59 ± 0.89	23.1 ± 0.4	35.58 ± 2.58	34.22 ± 2.25	P1 < 0.001	P2 > 0.05	P3 < 0.05	P4 < 0.001
PBF (%)	26.04 ± 0.8	25.84 ± 0.91	37.52 ± 0.85	36 ± 1.5	P1 < 0.001	P2 > 0.05	P3 < 0.01	P4 < 0.001
Waist (cm)	73.44 ± 2.97	73.08 ± 2.81	121.12 ± 4.37	118.56 ± 4.11	P1 < 0.001	P2 > 0.05	P3 < 0.05	P4 < 0.001
Hip (cm)	79.08 ± 4.44	78.68 ± 3.94	130.04 ± 3.04	129.6 ± 2.94	P1 < 0.001	P2 > 0.05	P3 > 0.05	P4 < 0.001
Resting HR (beat/min)	75.4 ± 3.3	75.8 ± 5.2	77.8 ± 2.9	77 ± 4.8	P1 > 0.05	P2 > 0.05	P3 > 0.05	P4 > 0.05
SBP (mmHg)	110 ± 7.2	112 ± 5.8	130 ± 11.98	128 ± 12.8	P1 < 0.001	P2 > 0.05	P3 > 0.05	P4 < 0.01
DBP (mmHg)	78 ± 5.4	79 ± 5.2	87 ± 9.85	88 ± 8.4	P1 < 0.001	P2 > 0.05	P3 > 0.05	P4 < 0.01
V O <sub>2</sub> max (ml/kg min)	32.00 ± 1.45	33.16 ± 0.93	21.72 ± 1.39	23.24 ± 1.09	P1 < 0.001	P2 < 0.01	P3 < 0.001	P4 < 0.001

applied to compare individual groups. Pearson's correlation analysis was used to determine the correlation among the parameters assessed. The p-value < 0.05 was considered statistically significant. All statistical analyses were done under supervision of the Institute of Statistical Studies and Research, Cairo University, Egypt.

## RESULTS

### Baseline characteristics of study population

The physical and clinical characteristics of the two groups, namely, normal-weight (n = 16) and obese (n = 15), enrolled in this study are displayed in Table 1. As expected, body mass index, percent body fat as well as waist and hip circumferences were significantly higher in the obese group compared with the normal-weight group (P1 < 0.001). Obese participants also had higher systolic blood pressure and higher levels of triglycerides, low-density lipoprotein (LDL) cholesterol, glucose and HbA1c (P1 < 0.001) in

spite of not being diabetic, the levels of high-density lipoprotein (HDL) cholesterol were lower (Table 2). Compared with the normal-weight group, endocrine functions were deranged in obese group as indicated by increased levels of insulin and leptin, while it exhibited decreased levels of adiponectin (Table 3) and nesfatin-1 (P1 < 0.001) (Table 4). In addition, the oxidative stress profile was also deranged as indicated by increased levels of GSSG and F2-Isoprostanes (Table 5) while a decreased level of GSH (P1 < 0.001) (Figure 1).

### Post exercise results

Responses to appetite questionnaires indicated less desire to eat, less perceived hunger, less craving for carbohydrates and lower scores on "how much food can you eat" questions. After the training period, the levels of different parameters in obese group remained significantly different

from those in normal-weight group. With regard to leptin level, it was extremely elevated in obese group as compared with normal - weight group before exercise (P < 0.001) but mildly elevated in obese group as compared with normal - weight group after exercise (P < 0.05).

The laboratory results obtained from the current study showed that 8 weeks of aerobic training increased V.O<sub>2</sub> max significantly in both groups, while some changes were exclusive in obese subjects as (Figure 2), decreased the obesity, atherogenicity and insulin resistance indices including BMI (P < 0.05), percent body fat (P < 0.01), waist circumference (P < 0.05) LDL-cholesterol (P < 0.05), fasting blood glucose (FBG) and HOMA-IR (P < 0.01) while they remain unchanged in control group. In addition, 8 weeks of training corrected the dysregulated endocrine homeostasis in obese subjects as indicated by lower mean plasma leptin (P < 0.01), insulin levels (P < 0.05) and higher mean nesfatin-1 levels (P < 0.01) relative to that of the level at the pre-test

**Table 2.** Metabolic markers in the study populations at baseline and after exercise.

Factor	Normal weight (16)		Obese (15)		P1	P2	P3	P4
	Before exercise	After exercise	Before exercise	After exercise				
HbA1c (%)	5.45 ± 0.38	5.39 ± 0.29	6.24 ± 0.29	6.21 ± 0.27	P1 < 0.001	P2 < 0.05	P3 < 0.05	P4 < 0.001
FBG (mg/dl)	91.16 ± 13.35	89.88 ± 11.11	119.7 ± 2.32	101.24 ± 5.24	P1 < 0.001	P2 < 0.05	P1 < 0.001	P1 < 0.001
T-cholesterol (mg/dl)	177.96 ± 28.71	173.08 ± 21.44	212.96 ± 13.14	203.88 ± 8.11	P1 < 0.001	P2 < 0.05	P1 < 0.01	P1 < 0.001
Triacylglycerols (mg/dl)	111.68 ± 23.56	107.08 ± 19.51	154.44 ± 18.62	146.28 ± 15.13	P1 < 0.001	P2 < 0.05	P1 < 0.05	P1 < 0.001
HDL-C (mg/dl)	57.16 ± 8.11	62.44 ± 3.68	37.00 ± 5.18	44.64 ± 5.78	P1 < 0.001	P2 < 0.05	P1 < 0.001	P1 < 0.001
LDL-C (mg/dl)	107.36 ± 18.62	105.16 ± 15.38	151.69 ± 20.49	141.96 ± 17.4	P1 < 0.001	P2 < 0.05	P1 < 0.05	P1 < 0.001
HOMA-IR	1.08 ± 0.33	1.00 ± 0.23	1.92 ± 0.40	1.54 ± 0.27	P1 < 0.001	P2 < 0.05	P1 < 0.001	P1 < 0.001

**Table 3.** Energy regulating hormones in the study populations at baseline and after exercise.

Factor	Normal-weight (16)		Obese (15)		P1	P2	P3	P4
	Before exercise	After exercise	Before exercise	After exercise				
Insulin (µU/ml)	4.76 ± 1.03	4.51 ± 0.78	6.88 ± 1.3	6.19 ± 1.3	P1 < 0.001	P2 > 0.05	P3 < 0.05	P4 < 0.001
Leptin (ng/ml)	10.05 ± 1.17	9.68 ± 1.71	14.45 ± 2.57	11.2 ± 2.31	P1 < 0.001	P2 > 0.05	P3 < 0.01	P4 < 0.05
Adiponectin (µg/ml)	15.01 ± 2.35	15.21 ± 2.22	8.95 ± 2.88	9.13 ± 2.26	P1 < 0.001	P2 > 0.05	P3 > 0.05	P4 < 0.001

**Table 4.** Appetite regulating hormone in the study populations at baseline and after exercise.

Factor	Normal-weight (16)		Obese (15)		P1	P2	P3	P4
	Before exercise	After exercise	Before exercise	After exercise				
Nesfatin-1 (ng/ml)	2.04 ± 0.7	2.07 ± 0.59	1.05 ± 0.25	1.3 ± 0.27	P1 < 0.001	P2 > 0.05	P3 < 0.01	P4 < 0.001

stage (before doing the exercise). Mean plasma adiponectin level was not significantly increased after exercise in both groups. Also, oxidative stress profiles have been improved in the experimental group after the regular training period as both GSSG ( $P < 0.05$ ) and F2-Isoprostanes ( $P < 0.001$ ) had significantly decreased while GSH had not changed by exercise. No differences within group were observed between the stages for the control group ( $P > 0.05$ ). However we further divided the

normal-weight participants into two groups according to each participant's individualistic leptin response to exercise: leptin responders (25%) defined when they showed decreased leptin level after exercise. Of notice, the baseline characteristics of the leptin responders showed deranged indices of obesity, atherogenicity, insulin resistance and oxidative stress including body weight, BMI, LDL-cholesterol, FBG, insulin, HOMA-IR, GSSG and F2-isoprostanes while after exercise in comparison by leptin response, they

have decreased in responders more than in non-responders ( $P < 0.05$ ). Whereas, HDL and the anorexigenic adipokine; nesfatin-1 have significantly increased ( $P < 0.05$ ).

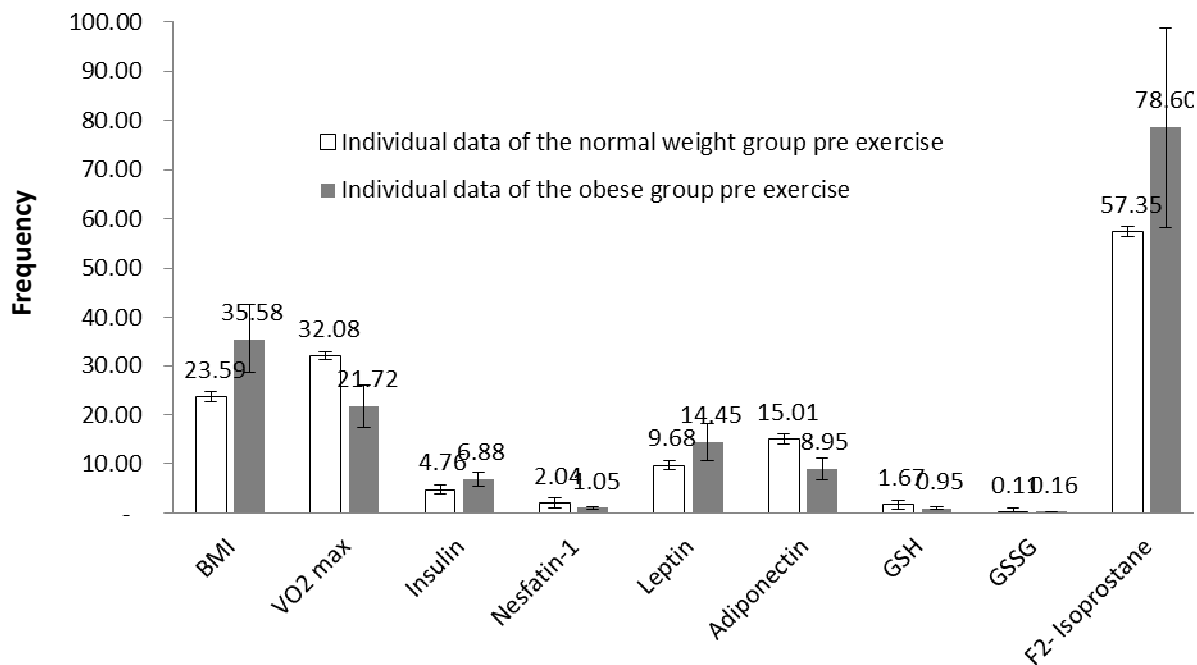
#### **Correlations between the changes in leptin, F2-Isoprostanes and the changes in various parameters**

In both the obese and the 25% responder normal-

**Table 5.** Oxidative stress markers in the study populations at baseline and after exercise.

Factor	Normal-weight (16)		Obese (15)		P1	P2	P3	P4
	Before exercise	After exercise	Before exercise	After exercise				
GSH ( $\mu\text{M}$ )	1.67 $\pm$ 0.27	1.71 $\pm$ 0.21	0.95 $\pm$ 0.26	0.96 $\pm$ 0.25	P1 < 0.001	P2 > 0.05	P3 > 0.05	P4 < 0.001
GSSG ( $\mu\text{M}$ )	0.11 $\pm$ 0.01	0.1 $\pm$ 0.01	0.16 $\pm$ 0.03	0.14 $\pm$ 0.02	P1 < 0.001	P2 > 0.05	P3 < 0.05	P4 < 0.001
F2-Isoprostanes (pg/ml)	57.35 $\pm$ 6.6	55.82 $\pm$ 5.77	78.6 $\pm$ 8.53	68.8 $\pm$ 5.36	P1 < 0.001	P2 > 0.05	P3 < 0.001	P4 < 0.001

P1: Significant difference between the normal-weight and the obese groups before exercise. P2: Significant difference between pre- and post- exercise in normal-weight group. P3: Significant difference between pre- and post- exercise in obese group. P4: Significant difference between the normal-weight and the obese groups after exercise.



**Figure 1.** Mean and Std error for normal weight and obese individuals pre exercise.

weight subjects,  $\Delta$ leptin levels after exercise were negatively correlated with  $\Delta$  V.O<sub>2</sub> max and  $\Delta$ HDL while positively correlated with  $\Delta$ F2-Isoprostanes

(Figure 3),  $\Delta$ HOMA-IR,  $\Delta$ LDL-cholesterol,  $\Delta$ W.C,  $\Delta$ percent body fat and  $\Delta$ BMI. For F2-Isoprostanes, there was negative correlation between  $\Delta$ F2-

Isoprostanes and  $\Delta$ nesfatin-1 (Figure 4) only in obese subjects. Furthermore,  $\Delta$ F2- Isoprostanes were positively correlated with leptin,  $\Delta$ HOMA-IR,

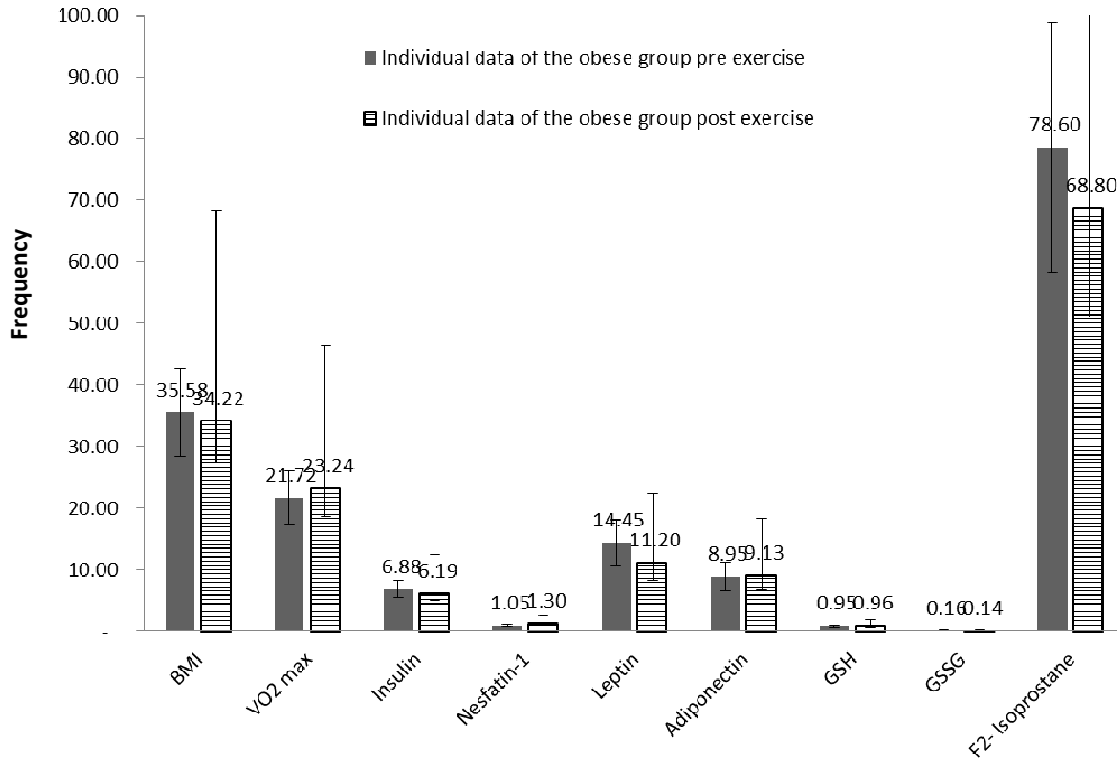


Figure 2. Mean and Std error for obese individuals pre and post exercise.

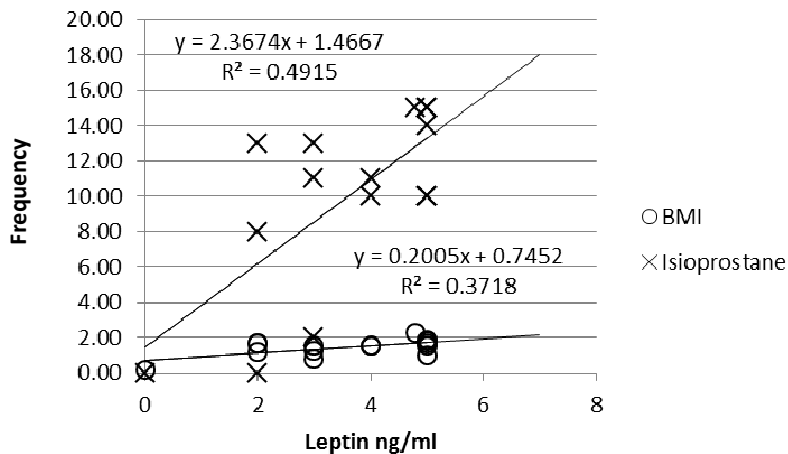


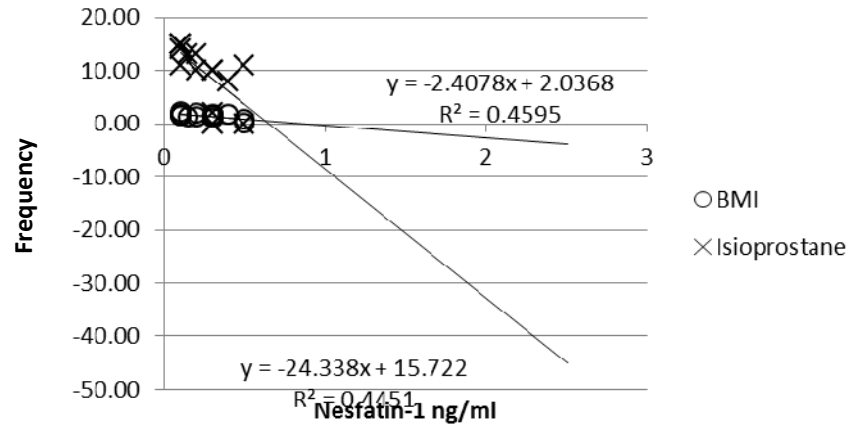
Figure 3. Correlation between the changes in leptin and the changes in both BMI and F2-Isoprostanes in obese women as a result of regular exercise.

ΔLDL-cholesterol, ΔW.C and ΔBMI in both the obese and 25% responder normal-weight subjects.

**DISCUSSION**

The current study was performed on women due to the

higher percentage of fat possessed by females, as well as for confirming or refuting the findings by previous reports that the hormonal and appetite responses to exercise are strongly effective strategy in men, but much less so in women (Donnelly et al., 2003). After the training period, the results of the current study revealed that the levels of different parameters in obese women



**Figure 4.** Correlation between the changes in nesfatin-1 and the changes in both BMI and F2-Isoprostananes in obese women as a result of regular exercise.

remained significantly different from those in normal-weight women. However, a significant within group differences were found after 8 weeks of aerobic training relative to the level at the pre-test stage as shown in the plasma levels of energy and appetite regulating hormones and oxidative stress markers in the experimental group and 25% of the normal-weight group (we called them leptin responders). No within-group differences were observed between pre- and post-test stages for 75% of the control group ( $P > 0.05$ ). Interestingly, plasma levels of nesfatin-1, HDL and  $VO_2$  max have increased after exercise in the obese and leptin responders.

In the present study, leptin and insulin, the two hormones which physiologically suppress appetite (Friedman and Halaas, 1998), were found to be significantly increased in the obese group compared with the control group. This finding is in accordance with previous studies which reported leptin and insulin resistance in addition to the relevant excessive energy intake in obese individuals. In the current study, 8 weeks exercise training resulted in a significant decrease in plasma leptin and insulin levels in the obese individuals and in 25% of the normal-weight group (leptin responders) compared to the rest of the control group [leptin level was extremely elevated in obese group as compared with normal-weight group before exercise ( $P < 0.001$ ) but mildly elevated in obese group as compared with normal-weight group after exercise ( $P < 0.05$ ).

There are several reports with similar findings; reporting the association between physical exercise and the plasma level of insulin and leptin (Konishi et al., 2011). Although investigators showed that lower concentrations of leptin and insulin stimulate appetite and energy intake and suppress energy expenditure (Benoit et al., 2004), the results of the current study demonstrated that there were significant decrease in BMI ( $P < 0.05$ ), percent body fat ( $P < 0.01$ ), waist

circumference ( $P < 0.05$ ) in obese subjects after 8 weeks exercise training and they were positively correlated with the decrease in plasma leptin level. One possible explanation for the current findings is that the relationship between leptin/insulin concentrations and energy intake is the familiar inverted U in which both low and very high concentrations of leptin/insulin stimulate food intake, but moderate circulating levels, which could be achieved by regular exercise training as indicated by this study, have a suppressive effect. The results of the present study indicated that ergometer exercise at moderate intensity did not change plasma adiponectin concentrations in both studied groups. Our results are in concurrence with Hulver et al. (2002) who found that adiponectin is unaltered with exercise training despite enhanced insulin action.

In this study, we examined the plasma level of nesfatin-1 which is an anorexogenic adipokine that inhibits food intake and regulates blood glucose in time-, dose- and insulin-dependent manners (Oh-I et al., 2006; Nakata et al., 2011). The present study found that nesfatin-1 has significantly increased after an 8 week supervised exercise program in the groups of both leptin responders and obese women. This finding came along with the responses to appetite questionnaires which indicated less desire to eat, less perceived hunger, less craving for carbohydrates and lower scores on "how much food can you eat" questions. These findings are in agreement with the results of Chaolu et al. (2011) who showed that four weeks of endurance training increased plasma nesfatin-1 level.

Shimizu et al. (2009) found that the intraperitoneal administration of nesfatin-1 inhibits food intake and thereby reduces body weight (Shimizu et al., 2009). After all, prevalence and complications of obesity create a more crucial understanding about the origin of energy imbalance and appetite dysregulation in obesity and knowing that obesity may induce systemic oxidative stress



and, in turn, oxidative stress is associated with an irregular production of adipokines (Esposito et al., 2006), which made it necessary to gain insight into whether the effects of exercise on hormonal and appetite responses are associated with improvement in oxidative stress. The last perspective was affirmed by measuring markers of oxidative stress pre- and post-exercise. In this sense, GSH/GSSG ratio has been studied as a sensitive marker of oxidative stress and has been found to decrease in obese subjects compared with non-leptin responder normal-weight subjects. However when the two groups carried out eight weeks of regular physical exercise, the mean plasma GSH was not significantly different from the pre-test stage whereas GSSG levels have significantly decreased in obese and leptin responder normal-weight subjects. GSH plays a multifunctional role in protecting biomolecules from oxidative damage during exercise so the findings in this study may suggest an acute compensatory response of the antioxidant defenses against the ROS production. In agreement with this compensation theory, Marin et al. (2011) found that GSH concentration was reduced immediately after a handball game. In addition to GSH/GSSG ratio, F2-Isoprostanes are considered to be the best available chemically stable biomarkers of oxidative stress induced lipid peroxidation *in vivo* (Roberts and Morrow 2000), that also present in detectable amounts in plasma and are unaffected by lipid content in the diet (Gopaul et al., 2000).

Consistent with a previous report (Schmitz et al., 2008), the present study found that the free F2-Isoprostanes in plasma were significantly lower following 8 weeks of exercise in obese and leptin responder normal-weight participants. However, some studies found decreased F2-Isoprostanes levels but only after acute excessive exercise (Nasca et al., 2010). The concurrence of decreased GSSG and F2-Isoprostanes levels after exercise confirms that chronic exercise training has been associated with a training-induced adaptive response against oxidative stress. Importantly, the changes in F2-Isoprostanes levels were positively correlated with those of leptin and negatively correlated with nesfatin-1 in both the leptin responders and obese individuals. The concomitant weight loss and alterations in leptin, nesfatin-1 and F2-Isoprostanes after eight weeks of moderate intensity exercise may imply the effects of physical activity on weight loss which involve alleviating the oxidative stress state thereafter correcting the hormone homeostasis resulting in changes in appetite, satiety and energy expenditure. In addition, it is noteworthy that before exercise, leptin responders and obese individuals in the current study showed relative perturbation of both glucose homeostasis and lipid profile while, then gets improved after exercise which adds further evidence that physical exercise might be one of the non-pharmacological approaches that can attenuate the development of metabolic syndrome.

## Conclusion

An 8 week, moderate-intensity, regular exercise program improved VO<sub>2</sub> max and oxidative stress and modified insulin, leptin and nesfatin-1 concentrations, particularly in obese women, who also achieved weight loss. These data suggest that exercise, having an antioxidant effect, could be a good strategy to manage elevated energy intake in obese women through the modification of leptin or nesfatin-1 levels.

## Conflict of interest

Authors declare that there are no conflicts of interest.

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