Effect of Diet Restriction on Serum Levels of Some Adipocytokins in A Rat Model of High Fat Diet Induced Obesity

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Abstract: Numerous adipocytokines are released by both fat cells and macrophages that infiltrate it in obesity. This study was designed to investigate the effect of obesity and diet restriction on serum levels of some adipocytokines by using high fat diet induced obesity rat model. Thirty adult male albino rats were used and randomly divided into three equal groups. Group 1 normal diet control group composition, group 2 obese rats in which obesity was induced by high fat diet (58% fat, 18% protein and 24% carbohydrates) for 16 weeks and group 3 fed on high fat diet (HFD) for 16 weeks then followed by diet restriction by feeding normal diet for 8 weeks. Body weight, abdominal circumference (AC), body Mass Index (BMI), serum levels of glucose, insulin, insulin resistance, lipid profile, adipocytokines [adiponectin, leptin, resistin, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP)] were estimated. Obtained results revealed that there was a significant increase in body weight, AC, BMI, glucose, insulin, insulin resistance, total cholesterol, triglyceride, Low density lipoproteins (LDLc), very low density lipoproteins (VLDLc), leptin, resistin, IL-6, TNF-α and CRP and a significant decrease in high density lipoproteins (HDLc) and adiponectin in group 2 when compared with group 1 and had significant improvement in group 3 compared with group 2. A significant correlation was found between BMI and all of AC, glucose, insulin, insulin resistance, total cholesterol, triglyceride, HDL, LDLc and VLDLc. Also, that there was found a significant correlation between insulin resistance and all of adiponectin, leptin, resistin, IL-6, TNF-α and CRP in obese group. In conclusion: The present study demonstrated that HFD was associated with central obesity, changes in serum levels of adipocytokins together with the presence of the criteria of metabolic syndrome such as dyslipidemia and type-2 diabetes mellitus. All these changes were improved by caloric restriction.

Keywords: Obesity, Adipocytokines, High Fat Diet, Diet restriction.

I. INTRODUCTION

In obesity, excess adipocytes have accumulated to the extent that it may increase health problems and/or decrease life expectancy (Haslam and James, 2005). Obesity can be induced in animals by several methods, high fat diet is one of the common methods to make obese model in rats (Buettneret al., 2007). Adipocytokine(adipokine) is any protein that is formed and secreted by adipocytes. It has been found that adipocytokines secretion is changed in obesity, type-2 diabetes and metabolic syndrome (MS) (Bastard et al., 2006; Saillanet al., 2003 and Trayhurn and Wood, 2004). Obesity is characterized by a low-grade inflammatory condition in adipose tissue which synthesizes and secretes several adipokines that associated with insulin resistance and cardiovascular problems (Das, 2002). The pathophysiological link between obesity and the alteration of adipose tissue and its secretory functions in human diseases has been revealed (Barzilay et al., 2001).

The present study was carried out to investigate the effect of diet restriction on serum levels of some adipocytokins in obesity rat model.

II. MATERIALS AND METHODS

Thirty healthy adult male albino rats weighing 155-177 g were obtained from the animal house of Faculty of Veterinary Medicine - Zagazig University. Rats were kept in steel wire cages (5/cage) under hygienic conditions. They were fed the commercial rodent chow with free access to water, kept at room temperature and were maintained on a 12 h light/dark cycle. Rats were adapted to the new environment for one week before the experiment going on. The animal experiments were approved by the Institutional Research Board.

Rats were randomly divided into three equal groups. Group 1 [normal control (n=10 rats)]: Rats were fed on normal chow diet which was consisted of (5% of weight of diet derived from fat, 18% from proteins, and 77% from carbohydrates) for 16 weeks (Svegliati-Baroni et al., 2006), group 2 [High fat diet feeding (n=10 rats)]: Rats were fed on high fat diet which was consisted of (58% of weight of diet derived from fat, 18% from protein, and 24% from carbohydrates) (Svegliati-Baroni et al., 2006). Group 3 [High fat diet induced obese group followed by diet restriction (n=10 rats)]: Rats were fed on high fat diet for 16 weeks then followed by diet restriction by feeding normal chow diet. In this group, the rats were fed on normal chow diet which was consisted of (5% of weight of diet derived from fat, 18% from proteins, and 77% from carbohydrates) for 8 weeks.

Measurement of body weight and length:

Each rat was put in closed plastic container and was weighed at the first and the last day of the experiment. The results were written in a record for each rat.

Body length was taken as the distance from the nose tip to the anus at the start and the end of experiment. Approximately the same amount of stretching of the body for measurement was obtained in all rats as one person held the rat throughout the data collections (Novelli et al, 2007).

Calculation of Body Mass Index [BMI]:

MBI = body weight (gm) / length² (cm²). The cutoff value of obesity is BMI more than 0.68 gm/cm² (Novelli et al, 2007).

Measuring of abdominal circumference (AC):

A plastic tape was used to measure the abdominal circumference at the largest zone of the rat’s abdomen (Gerbaix et al., 2010).
Calculation of Insulin resistance (HOMA-IR)

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula (Matthews et al. in 1985).

\[ \text{HOMA-IR} \times \text{glucose (mg/dl) /405} \]

Blood sampling

Retro-orbital venousplexus blood samples were obtained then serum was separated by allowing the samples to clot then centrifuged at 3000 rpm for 20 minutes, kept at (-20o c) and used to measure the serum levels of glucose, insulin, lipids profile and Adipocytokines [adiponectin, leptin, resistin, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP)].

Measurement of serum glucose and insulin:

Serum glucose was estimated as described by Tietz (1995) using specific glucose kit (Bioscience, Egypt) and analyzed by spectrophotometers device (URIT-810, China). Insulin was measured by enzyme amplified sensitivity immunoassay (EASIA) as described by Temple et al., 1992 using specific insulin kit (BioSource Belgium) and analyzed by spectrophotometers device.

Measurement of serum lipids profile:

Total cholesterol (TC) and triglycerides (TG) were measured by enzymatic colorimetric method described by Tietz (1995) using specific cholesterol and triglycerides kits (Spinreact Spain) and analyzed by spectrophotometers device. High density lipoproteins (HDLc) was measured by precipitating reagent method described by Tietz (1995) using HDLc precipitating reagent kit (Spinreact, Spain) and analyzed by spectrophotometers device. Low density lipoproteins (LDLc) and very low density lipoproteins (VLDLc) were estimated by using Friedewald et al. (1972) formula.

\[
\text{LDLc} = \frac{\text{TC} - \text{HDLc} - \left( \frac{\text{TG}}{5} \right)}{\text{VLDLc}} = \frac{\text{TG}}{5}
\]

Measurement of serum Adipocytokines (Adiponectin, IL-6, TNF-alpha, Resistin, Leptin and CRP):

Adipocytokines were estimated by enzyme-linked immunosorbent assay (ELISA) method described by Engelall E. and Perlmann P. (1971) by using Adiponectin (ADP) ELISA Kit (Sunred Bio, China), IL-6 (Interleukin-6) ELISA Kit (AviBion, Korea), Resistin ELISA Kit (Koma Biotech, Korea), TNF-Alpha ELISA Kit (Koma Biotech, Korea), Leptin ELISA kit (DRG International Inc., USA) and C - reactive protein (CRP) ELISA Kit (Sunred Biological, China) analyzed by ELISA Microplate Reader device (Tecan, Austria).

Statistical Analysis:

Results were presented as mean ± SD for and analyzed using version 18 SPSS program (SPSS Inc. Chicago, IL, USA). One way Analysis of variance (ANOVA) was used followed by student- least significant differences (LSD) test to compare statistical differences between groups. P value less than 0.05 was considered to be significant. Pearson’s test was done to detect correlations between parameters.

III. RESULTS

As shown in table (1) and fig. (1 to 17) it was found that there was a significant (p<0.001) increase in body weight, AC, BMI, glucose, insulin, insulin resistance, total cholesterol, triglyceride, LDLc, VLDLc, leptin, resistin, IL-6, TNF-α and CRP and a significant (p<0.001) decrease in HDLc and adiponectin in obese group compared with the control group. However, a significant (p<0.001) reduction in body weight, AC, BMI, glucose, insulin, insulin resistance, total cholesterol, triglyceride, LDLc, VLDLc, leptin, resistin, IL-6, TNF-α and CRP and a significant (p<0.001) increase in HDLc and adiponectin were observed in diet restriction group compared with obese group. A significant correlation was found between BMI and all of AC, glucose, insulin, insulin resistance, total cholesterol, triglyceride, HDLc, LDLc and VLDLc. Also, that there was a significant correlation between insulin resistance and all of adiponectin, leptin, resistin, IL-6, TNF-α and CRP in obese group as in fig. (18 to 32).

IV. DISCUSSION

The present study was designed to evaluate the effect of obesity induced by high fat diet (HFD) on some adipocytokines in rats. Animals fed on high fat diet which was consisted of 58% of weight of diet derived from fat, 18% from protein, and 24% from carbohydrates (Svegliati-Baroni et al., 2006) showed significant increase in BMI which indicates the presence of an overall obesity and significant increase in AC which indicates the presence of central obesity and these results are in agree with the findings of Amin et al., 2011, Buettner et al., 2007, Kanazawa et al., 2003. body-mass index (BMI) is most commonly used formula for estimating the body fat in epidemiological studies (Kopelman, 2000).

There is a strong relationship between BMI and several human diseases including type-2 diabetes (Zhang et al., 2008). This finding was proved in the present study which showed a significant increase in serum levels of glucose and insulin together with the presence of a significant increase in insulin resistance.

Suppressed insulin action is associated with hyperlipidemia that led to increased storage of lipids in insulin target tissues (e.g. muscle, liver and adipose tissue) and also increased plasma free fatty acids (FFA) and triglyceride (Frayn, 2002). FFA's have a deleterious effect on insulin uptake by the liver and hepatic glucose release observed in obesity.

The present study revealed that high fat diet induced obesity was associated with a significant increase in total cholesterol, triglyceride, LDL-c and VLDL-c serum levels beside a significant decrease in HDL-c serum level. These findings indicate the presence of dyslipidemia which is considered as one of the criteria of metabolic syndrome.

Obesity leads to many health problems including dyslipidemia (Jabeen et al., 2011). This dyslipidemia leads to many complications including atherosclerosis with cardiovascular problems, neuropathy, cerebrovascular accidents and respiratory affection (Becker et al., 2002). Oxidative stress is a primary risk factor for the development of dyslipidemia in obesity and may also contribute to various detrimental consequences of obesity (Diniz et al., 2005).

In obese subjects, oxidative stress and subclinical inflammation have been found in adipose tissue and these may be responsible for obesity-related metabolic syndrome, insulin resistance and diabetes mellitus (Gariballa et al., 2014).

Excess fatty tissue leads to excess or deregulated production of adipocytokines, which produce a chronic low-grade inflammatory condition and can lead to obesity-related metabolic diseases through alteration in glucose and lipid homeostasis (Halberg et al., 2008 and Jung and Choi, 2014).
The present study showed that obesity induced experimentally in male rats by giving a high fat diet resulted in significant increase in serum levels of leptin, resistin, TNF-α and IL-6 while there was a significant decrease in serum levels of adiponectin.

Adipose tissue considered the main energy store in the body and it is also an active endocrine organ (Kershaw and Flier, 2004). Obese patients also have high macrophage infiltration in fatty tissue in comparison to lean subjects that affects the production of various inflammatory molecules as TNF-α, IL-6 and adiponectin that play a definitive role in the occurrence of dyslipidemia (Jung and Choi, 2014).

In obese patients, there is a positive correlation between macrophage infiltration in visceral adipose tissue and plasma triglyceride level and a negative correlation between that infiltration and plasma HDL cholesterol level (Cancello et al., 2006). Also, there are positive correlations between a macrophage-specific marker (CD68) in subcutaneous adipose tissue and plasma free fatty acid and LDL levels but there is a negative correlation between this marker and HDL levels (Huber et al., 2008).

Several adipokines also enhance lipolysis and decrease the clearance of lipoproteins in adipose tissue (Hardardottir et al., 1994 and Yang et al., 2008)). IL-6 and TNF-α decrease the activity of lipoprotein lipase (LPL), a key enzyme in the regulation of lipoproteins metabolism in adipose cells (Yang et al., 2008).

Plasma TNF-α is high in hyperlipidemic subjects and there is positive correlation between its level and VLDL levels (Jovinge et al., 1998). TNF-α also generates a high triglyceride levels in bacteria infected-animals (Rouzer and Cerami, 1980). These effects are due to stimulation of triglyceride formation in the liver (Feingold et al., 1989) as well as inhibition of LPL (Kawakami and Cerami, 1981).

In addition, TNF-α diminishes hepatic insulin signaling in animals that increases the hepatic apolipoprotein B100-containing VLDL production (Qin et al., 2008). IL-6 is also associated with high plasma triglyceride levels. Patients with hypertriglyceridemia have a high IL-6 and TNF-α generation (Jonkers et al., 2002 and Nappo et al., 2002).

Adiponectin decreases VLDL and plasma triglyceride levels via activation of LPL (Berneisand Krauss, 2002). It also stimulates fatty acid and glucose oxidation through stimulation of adenosine monophosphate-activated protein kinase (AMPK) (Yamauchi et al., 2002). Decreased adiponectin levels, as was proved in the present study and reported by many other studies, are associated with dyslipidemia and cardiovascular problems (Okamoto et al., 2006).

Leptin is produced primarily by adipose tissue and controls the energy balance via suppressing appetite and stimulating energy expenditure (Friedman and Halaas, 1998). The inflammatory cytokines stimulate leptin formation and secretion, which plays a major role in maintaining the chronic inflammatory condition that is seen in obesity (Paz-Filho et al., 2012).

Resistin level also increases with obesity and a significant correlation between its level and insulin resistance has been shown (Satoh et al., 2004). On the other hand, absence of resistin secures mice from hyperglycemia by stimulating AMPK and decreasing the hepatic gluconeogenic enzymes expression (Banerjee et al., 2004).

The present study showed that high fat diet induced obesity was associated with a significant increase in serum level of C-reactive protein (CRP) which is used as an indicator of chronic low grade inflammation. This level was significantly decreased by caloric restriction. These finding are in agreement with those of Black et al. (2004) who postulated that the chronic inflammatory state can be observed by higher CRP levels.

CRP is an acute-phase plasma protein, which contributes in the systemic response to inflammation (Dimitrov et al., 2014). CRP is formed by hepatocytes, macrophages, smooth muscle cells and fat cells (Funtuzzi, 2005). Increased CRP levels have been shown to be associated with increased the risk of cardiovascular disease, diabetes mellitus, and multiple components of the metabolic syndrome (Black et al., 2004).

The present study showed that caloric restriction resulted in weight loss which was associated with a significant decrease in serum glucose, insulin, insulin resistance, total cholesterol, triglyceride, LDLc, VLDLc, leptin, resistin, IL-6, TNF-α and CRP and significant increase in HDLc and adiponectin when compared with obese group. These findings are in agreement with those of many studies which demonstrated that weight reduction improves many of the medical complications associated with obesity, including insulin resistance, serum levels of glucose, insulin, adiponectin, TNF-α, leptin, resistin, IL-6, and CRP as well as the lipid profile (Anderlová et al., 2006; Margoni et al., 2011 and Reinherz et al., 2005).

CONCLUSION

The present study revealed that high fat diet induced obesity in a rat model is associated with a state of chronic low grade inflammation as indicated by the significant increase in serum levels of TNF-α, IL-6 and CRP. Also, there was a significant increase in serum leptin and resistin levels and a significant decrease in serum adiponectin level together with the presence of hyperinsulinemia, hyperglycemia, insulin resistance (i.e type-2 diabetes) and dyslipidemia. All these changes indicate the presence of metabolic syndrome (MS) which was found to be significantly ameliorated by caloric restriction.

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Table 1: The mean values ± SE of all parameters studied and its comparison in different groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (n=10) Control</th>
<th>Group 2 (n=10) Obese</th>
<th>Group 3 (n=10) Obese + Diet restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (gm)</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>165.3 ± 2.024846</td>
<td>166.9 ± 1.81523</td>
<td>169.1 ± 1.409404</td>
<td></td>
</tr>
<tr>
<td>Final body weight (gm)</td>
<td>280.6 ± 3.425395</td>
<td>420.8 ± 5.838992</td>
<td>333.2 ± 3.72446</td>
</tr>
<tr>
<td>BMI (gm/cm²)</td>
<td>0.573 ± 0.018396</td>
<td>0.848 ± 0.01936</td>
<td>0.687 ± 0.018396</td>
</tr>
<tr>
<td>Abdominal circumference(cm)</td>
<td>16.7 ± 0.210819</td>
<td>21.15 ± 0.324418</td>
<td>18.2 ± 0.179161</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>83 ± 1.555556</td>
<td>219.4 ± 6.252308</td>
<td>114.8 ± 4.81715</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>24.87 ± 0.562633</td>
<td>46.18 ± 1.249573</td>
<td>33.92 ± 1.091471</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.093 ± 0.132777</td>
<td>25.075 ± 1.156035</td>
<td>9.584 ± 0.419138</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>67.745 ± 1.20126</td>
<td>182.15 ± 3.89387</td>
<td>111.572 ± 5.57419</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>87.83 ± 2.291744</td>
<td>144.48 ± 4.586098</td>
<td>107.18 ± 3.807232</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>33.838 ± 0.877187</td>
<td>26.419 ± 0.590351</td>
<td>30.244 ± 0.664127</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>16.341 ± 0.456869</td>
<td>126.835 ± 3.666232</td>
<td>60.092 ± 5.463526</td>
</tr>
<tr>
<td>VLDLc (mg/dl)</td>
<td>17.566 ± 0.45835</td>
<td>28.896 ± 0.91722</td>
<td>21.436 ± 0.761446</td>
</tr>
<tr>
<td>Adiponectin (ng/dl)</td>
<td>6.339 ± 0.120794</td>
<td>3.958 ± 0.249284</td>
<td>5.368 ± 0.135827</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>3.272 ± 0.208426</td>
<td>9.509 ± 0.66459</td>
<td>5.968 ± 0.515641</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>8.295 ± 0.313157</td>
<td>23.085 ± 0.673044</td>
<td>12.873 ± 0.698086</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>46.541 ± 0.545363</td>
<td>59.81 ± 0.65487</td>
<td>50.996 ± 0.633714</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>7.813 ± 0.712298</td>
<td>21.885 ± 1.284713</td>
<td>13.417 ± 0.820638</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.0416 ± 0.004741</td>
<td>0.152 ± 0.010864</td>
<td>0.0823 ± 0.002794</td>
</tr>
</tbody>
</table>

Fig 1: Final weight in studied groups
Fig 2: BMI in studied groups
Fig 3: Abdominal circumference in studied groups
Fig 13: Leptin in studied groups

Fig 14: TNF-α in studied groups

Fig 15: IL-6 in studied groups

Fig 16: Resistin in studied groups

Fig 17: CRP in studied groups

Fig 18: Correlation between BMI and Abdominal circumference (AC) (cm) in group 2

Fig 19: Correlation between BMI and Glucose level in group 2
**Fig (20):** correlation between BMI and Insulin level in group 2

**Fig (21):** correlation between BMI and HOMA-IR level in group 2

**Fig (22):** correlation between BMI and Total cholesterol level in obese group

**Fig (23):** correlation between BMI and Triglyceride level in obese group

**Fig (24):** correlation between BMI and HDLc level in obese group

**Fig (25):** correlation between BMI and LDLc level in obese group
Fig (26): correlation between BMI and VLDLc level in obese group

Fig (27): correlation between ADP and HOMA-IR levels in obese group

Fig (28): correlation between TNF-α and HOMA-IR levels in obese group

Fig (29): correlation between Leptin and HOMA-IR levels in obese group

Fig (30): correlation between Resistin and HOMA-IR levels in obese group

Fig (31): correlation between IL-6 and HOMA-IR levels in obese group
Fig(32): correlation between CRP and HOMA-IR levels in obese group

**References**


