Evaluating the levels of asprosin, adiponectin, and a number of physiological variables in obese women in the city of Kirkuk.

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Abstract:
The current study involved measuring the concentration of asprosin and adiponectin hormones, as well as cardiac troponin I and creatine kinase, in 100 samples of women divided as follows: 50 blood samples from obese women, 30 blood samples from thin women, and 20 blood samples from women of ideal weight. Their ages ranged between 25 and 45 years. This study was conducted from November 2021 to the end of April 2022. Blood samples were taken early in the morning after fasting for at least 6-8 hours, excluding pregnant and ill individuals. Statistical analysis showed a significant increase (P≤0.01) in asprosin concentration in obese women (5.82 ± 0.83) ng/ml compared to the control group (3.27 ± 0.40) ng/ml. Meanwhile, there was no significant difference in asprosin concentration in thin women (3.10 ± 0.22) ng/ml compared to the control group (3.27 ± 0.40) ng/ml. As for adiponectin, there was a significant decrease at (P≤0.05) in the adiponectin concentration when comparing obese women (8.11 ± 0.57) mg/L with the control group (13.22 ± 1.02) mg/L. In contrast, the results showed a significant increase at the level of (P≤0.05) in the adiponectin concentration when comparing thin women (14.56 ± 7.89) mg/L with the control group (13.22 ± 1.02) mg/L.

The statistical analysis also showed no significant difference in the concentration of troponin I in the blood serum of obese women (0.18 ± 0.150) ng/ml and the blood serum of thin women (0.02 ± 0.108) ng/ml compared to the control group (0.03 ± 0.115) ng/ml. However, the study results revealed a significant increase (P≤0.05) in the concentration of creatine kinase in the blood serum of obese women (56.83 ± 72.22) UI/L compared to the control group (17.6 ± 51.22) UI/L, whereas no significant difference was noticed in the concentration of creatine kinase in the blood serum of thin women (27.08 ± 50.81) UI/L compared to the control group (17.6 ± 51.22) UI/L.

Keywords: Obesity, Asprosin hormone, Adiponectin hormone, Troponin, Creatine kinase.
Introduction:

Obesity is a global health issue as declared by the World Health Organization, predicting it to become the epidemic of the 21st century. Women, in general, tend to have higher obesity rates than men, typically due to excessive food intake, decreased physical activity, and genetic predisposition (Wael et al., 2022). The primary cause of obesity is an imbalance between consumed and expended energy. It is defined as a metabolic disorder that results in increased fat storage, elevated cholesterol levels, and the most common and easiest way to measure obesity is through calculating the Body Mass Index (BMI), a ratio of weight to height used to classify overweight and obesity among individuals and adult populations (Adewoyin et al., 2015).

Obesity contributes to the onset of many serious diseases, including insulin resistance, hypertension, and can lead to type II diabetes, coronary artery disease, stroke, non-alcoholic fatty liver disease, and metabolic syndrome (Saklayen, 2018). Obesity is closely linked to the development and occurrence of cardiovascular diseases, including coronary artery disease (CAD) and non-ischemic heart disease (inadequate oxygen supply). Obesity is often accompanied by damage to the heart muscle (Koliaki et al., 2019). Therefore, obese individuals who have elevated troponin enzyme concentrations are most susceptible to the risk of heart disease (Tang et al., 2020).

Excessive accumulation of adipose tissue in obesity causes an imbalance in the secretion of pro-inflammatory and anti-inflammatory adiponectin, leading to hyperinsulinemia, insulin resistance, and other obesity-related disturbances (Czech, 2017). The reduced storage capacity of individual fat cells thus leads to fat deposition in organs, including visceral adipose tissues, liver, and muscles.

Recently discovered hormones such as asprosin, discovered by Romere et al. in 2016, and their influence on obesity are of interest. Asprosin, a protein hormone produced in the lobes of white adipose tissue (WAT), stimulates the liver to release glucose into the bloodstream. It plays a crucial role in maintaining normal brain metabolism and survival, and significantly influences glucose metabolism, with higher hormone levels observed in patients with insulin resistance (Romere et al., 2016).

Adiponectin is primarily produced and secreted by adipose tissues and is a regulatory peptide hormone mainly released by fat cells, heart muscle cells, endothelial and stromal cells. It operates through two primary receptors, AdipoR1 and AdipoR2, forming an "Adiponectin system" that actively exercises its cellular mechanisms and responses in target cells to regulate various metabolic processes (Aljafary & Al-Suhaimi, 2020).

Another study found that people with morbid obesity have higher CK levels as a result of increased muscle stress; the reason is the decreased blood flow in case of weight increase related to the diameter of the blood capillaries, leading to fat leakage into the muscles, which can eventually lead to reduced blood circulation or reduced oxygen. Men were found to have more muscular
symptoms, higher weight, and BMI than the control group with normal CK levels as CK is primarily derived from skeletal muscles (Lilleng et al., 2013).

The present study aims to evaluate the concentration of asprosin hormone, adiponectin hormone, and the concentrations of cardiac enzymes such as hs-Troponin I and creatine kinase (CK) in the blood serum of obese and thin women compared to a control group of healthy women.

**Materials and Methods**

Blood was collected by using a syringe (5ml) from a venous source and put in a gel tube without anticoagulant for serum analysis. The blood components in the tube were separated using a centrifuge at a speed of 3000 rpm for 15 minutes. The remaining serum was then drawn with a micropipette, transferred to an Eppendorf tube, and stored at -20°C for subsequent hormonal assays.

**Body Mass Index (BMI) Calculation:**

This measure includes the weight and height of all the samples to calculate the BMI, which is the ratio of weight in kilograms to the square of height in meters (kg/m²). This is achieved using a tape to measure the height and a scale to measure weight according to the method mentioned by (Aminian et al, 2018).

**Estimation of ASP Concentration in Serum:**

The primary principle for estimating the concentration of the asprosin hormone was to use a ready-made test kit from BT-LAB. This test relies on the Enzyme-Linked Immunosorbent Assay (ELISA) method. The plate was pre-coated with antibodies for human asprosin. The asprosin in the sample binds to the coated antibodies in the wells. Then, biotinylated asprosin antibodies are added and bind to the asprosin in the sample. Afterwards, Steptavidin-HRP is added and binds to the Biotinylated Asprosin antibody. After incubation, Streptavidin-HRP is washed, and a substrate solution is added, changing color according to the quantity of human asprosin. The reaction is stopped by adding the Stop Solution to the reaction wells, then read at a wavelength of 450 nm using a microplate reader.

**Estimation of ADP Concentration in Serum:**

The primary principle for estimating the concentration of the adiponectin hormone was to use a ready-made test kit from BT-LAB. The method is similar to that used in the asprosin assay.

**Estimation of Cardiac Troponin I Concentration:**

The concentration of troponin enzyme was estimated using a test kit from the American company Fine care on the Fine care machine. This method, based on Fluorescence Immunoassay Technology, uses a rapid quantitative test through a sandwich immuno-detection method.

**Estimation of Creatine Phospho Kinase Concentration:**

This estimation is based on color changes. 10 µL of the studied serum sample was placed on a FUJI DRI-CHEM CPK slide. The sample was incubated at a
temperature of 37 degrees Celsius, which stimulates a reaction of creatine phosphate ADP·ATP. This reduces nitrotetrazolium blue (NTB) by action of synchronous enzymes such as hexokinase and glucose-6-phosphate dehydrogenase (G6PD) to form a purple dye diaphormase. The increase in absorption is measured by the generated dye at 540 nm, and CPK activity is calculated according to the composite formula.

**Statistical Analysis:**

The results were statistically analyzed using the statistical program (Minitab) and an Analysis of Variance (ANOVA) test was applied to compare means using the Duncun Multiple Range test at a probability level (P≤0.01) and (P≤0.05) to determine significant differences between the groups (Al-Rawi, 2000).

**Results and Discussion**

**Estimation of Asprosin hormone concentration in blood serum**

The results shown in Table 1 indicate a significant increase (P≤0.01) in the concentration of Asprosin hormone in the blood serum of obese women (5.82 ± 0.83 ng/ml) compared to the control group (3.27 ± 0.40 ng/ml). On the other hand, there were no significant differences in the concentration of Asprosin hormone in the blood serum of thin women (3.10 ± 0.22 ng/ml) compared to the control group (3.27 ± 0.40 ng/ml).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group</th>
<th>Obese Group</th>
<th>Thin Group</th>
<th>P-Value @0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP (ng/ml) Mean ± Std.</td>
<td>3.27 ± 0.40b</td>
<td>5.82 ± 0.83**a</td>
<td>3.10 ± 0.22b</td>
<td>0.001</td>
</tr>
</tbody>
</table>

- **Estimation of Asprosin concentration in blood serum**

The results shown in Table (1) indicate a significant increase (P≤0.01) in the concentration of Asprosin hormone in the blood serum of obese women (5.82 ± 0.83 ng/ml) compared to the control group (3.27 ± 0.40 ng/ml). On the other hand, no significant difference was observed in the concentration of Asprosin hormone in the blood serum of thin women (3.10 ± 0.22 ng/ml) compared to the control group (3.27 ± 0.40 ng/ml).

Hormonal variations are crucial physiological indicators of the body’s natural and environmental equilibrium, i.e., the body’s homeostasis in terms of energy balance and body weight maintenance. Consequently, the current study revealed an increase in the concentration of Asprosin hormone (P≤0.01) in obese women compared to the control group, as illustrated in Table (1).

The causes of obesity could be pathological, such as hormonal imbalances, genetic, excessive food intake (poor diet) without physical effort like sports to burn excess calories leading to weight gain, or due to depression cases as some depression cases cause excessive appetite opening.
This result of our current study aligns with the finding of Beutler et al., 2018, which highlighted that Asprosin clearly rises, leading to an increase in appetite and a change in the energy balance between the consumed and lost energies, thus resulting in an increase in body weight and insulin resistance. Given that Asprosin directly works on nerve cells in the hypothalamic region, causing them to be gradually and slowly activated, leading to a gradual increase in appetite and food consumption. These nerve cells are essential for regulating food intake and energy balance. Also, our study results agree with the study of Ugur et al., 2019, which proved that the relationship between obesity and Asprosin is direct, as Asprosin level increases in people suffering from obesity. Asprosin level varies according to the biological clock rhythm; after fasting overnight, its level significantly rises in humans, mice, and rats, then decreases after eating. Injection of recombinant Asprosin also leads to increased blood glucose and hyperinsulinemia (Mazur, 2021). The results of our current study are also consistent with the study of Cantay et al., 2022, conducted on patients with morbid obesity, and their results of Asprosin hormone and immune factors were high in them. When they underwent laparoscopic gastric sleeve surgery to treat morbid obesity, blood Asprosin levels significantly decreased 6 months after bariatric surgery. Thus, levels of Asprosin hormone in adipose tissue are a potential risk factor in causing obesity.

As for the concentration of Asprosin hormone in the blood serum of thin women, as shown in Table (1), no significant difference was observed, and there was a slight decrease when compared to the control group. This may be due to the intake of a small amount of food with increased physical activity or genetic causes or psychological changes that may affect metabolism. Since Asprosin is primarily secreted by adipocytes of white adipose tissue during hunger, that is, it is a regulator in metabolic balance, we find that thin women do not have an increase in the concentration of Asprosin because adipocytes are less than normal, unlike obese women, the ratio of Asprosin increases due to a higher proportion of adipocytes.

**Estimation of Adiponectin concentration in blood serum**

The results in Table (2) show a significant decrease in the concentration of Adiponectin hormone ($P \leq 0.05$) when comparing obese women ($8.11 \pm 0.57$ mg/L) with the control group ($13.22 \pm 1.02$ mg/L). In contrast, the results showed a significant increase in the concentration of Adiponectin hormone at ($P \leq 0.05$) when comparing thin women ($14.56 \pm 7.89$ mg/L) with the control group ($13.22 \pm 1.02$ mg/L).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Obese</th>
<th>Thin</th>
<th>P-Value @0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (mg/L)</td>
<td>13.22 ± 1.02a</td>
<td>8.11 ± 0.57*c</td>
<td>14.56 ± 7.89*b</td>
<td>0.119</td>
</tr>
</tbody>
</table>
Table (2) demonstrates the concentration of adiponectin hormone in the blood serum of the groups studied. Differing letters indicate a significant difference in the groups under study.

Adiponectin increases the burning of fatty acids and energy consumption, leading to a decrease in the content of liver and skeletal muscles of triglyceride with a consistent increase in insulin sensitivity. This concurs with the study by Doumatey et al. (2012), which suggested that adiponectin levels inversely correlate with total fat mass under almost all physiological conditions. Thus, adiponectin secretion is predominantly determined by the quality of adipose tissues, not the quantity.

Furthermore, the present study aligns with some studies indicating that adiponectin levels decrease in patients suffering from insulin resistance, type II diabetes, obesity, or cardiovascular diseases. The decrease in adiponectin levels in plasma in these pathological conditions aligns with the reduced gene expression of adiponectin in adipose tissues, and that the obesity-associated increases in adipocyte size are accompanied by decreased secretion and decreased levels of circulating adiponectin in the blood. This deficiency in blood leads to an increase in cellular fat content and insulin resistance in muscles, skeleton, and liver (Fu et al., 2005).

The current study results are also in line with the study by Kishida et al. (2014) which focused on the importance of adiponectin as a clinical biological indicator of obesity-related diseases. High levels of circulating adiponectin inversely correlate with body weight. Especially, low concentrations of circulating adiponectin and visceral fat accumulation cause a variety of diseases, including metabolic syndrome, type 2 diabetes, insulin resistance, hypertension, dyslipidemia, metabolic syndrome, hyperuricemia, atherosclerosis, coronary artery disease, stroke, peripheral artery disease, sleep apnea, non-alcoholic fatty liver disease, gastritis, inflammatory bowel disease, pancreatitis, osteoporosis, and various cancers (endometrial, postmenopausal breast, leukemia, colon, stomach, prostate). On the other hand, hyperadiponectinemia is associated with heart, kidney, and lung diseases. Routine measurement of adiponectin is highly recommended in patients with lifestyle-related diseases.

Our current study also agrees with the study by AL-Bazzi (2023), which found a significant decrease in the concentration of adiponectin hormone in obese women compared to those of normal weight, indicating that adiponectin concentration inversely correlates with the body mass index. As for the lean women in the current study results, it was noticed that the concentration of adiponectin in lean women increased when compared to the control group. Weight loss due to dietary restriction or calorie consumption as a result of physical activity leads to an increase in adiponectin hormone to maintain energy balance through the burning of fatty acids. This is consistent with the study by Baker et al. (2019), which indicated a comparison of increased adiponectin levels with weight loss leading to decreased skeletal muscle mass,
decreased muscle density, and weakened physical performance (Baker et al., 2019). Also, our study agrees with the study by Corbi et al. (2019), which indicated that adiponectin hormone concentration increases in people suffering from obesity, and performing physical exercises and balanced diet improves metabolic performance, which reduces body weight and thus, the adiponectin hormone rises as the body weight decreases - an inverse proportion.

- Estimation of cardiac Troponin I concentration in blood serum

The results in Table (3) show no significant difference in the concentration of Troponin I in the blood serum of obese women (0.150 ± 0.18 ng/ml) and lean women’s blood serum (0.108 ± 0.02 ng/ml) when compared with the control group (0.115 ± 0.03 ng/ml).

Table (3) illustrates the concentration of cardiac Troponin I in the blood serum of the studied groups.

- Different letters denote a significant difference in the studied groups.

Although obesity is associated with cardiovascular diseases, and the enzyme troponin is one of the regulatory enzymes present in heart tissues and is one of the main and most specific biomarkers for the early diagnosis of acute myocardial infarction and to assess the degree of heart muscle damage in non-cardiac diseases that can negatively affect the cells of heart muscle tissue (Chaulin., 2022), we do not find a noticeable increase in the concentration of the troponin enzyme. The increase was very slight and therefore there is no significant difference in our current study when comparing obese women with the control group. The obese group chosen for the research are healthy, and the concentration of Troponin enzyme increases during the first three hours from the onset of chest pain. But in our current study, this slight increase in obese women may be a sign of the beginning, and it is possible that the obese woman may suffer from a heart disease in the future if obesity is not properly addressed.

- Assessment of the concentration of Creatine Kinase (CK-MB) in blood serum

The results of the current study, illustrated in Figure (4), show a significant increase (P≤0.05) in the concentration of the enzyme Creatine Kinase (CK-MB) in the blood serum of obese women 72.22 ± 56.83 UI/L when compared with the control group 51.22 ± 17.6 UI/L. On the other hand, there was no significant difference in the concentration of Creatine Kinase (CK-MB) in the blood serum of lean women 50.81 ± 27.08 UI/L when compared with the control group 51.22 ± 17.6 UI/L.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Obese</th>
<th>Lean</th>
<th>P-Value @0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropo (ng/mL)</td>
<td>0.115 ± 0.03</td>
<td>0.150 ± 0.18</td>
<td>0.108 ± 0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>CK-MB(UI/L)</td>
<td>Mean ± Std</td>
<td>Mean ± Std</td>
<td>Mean ± Std</td>
<td>51.22±17.6 72.22 ± 56.83 50.81 ± 27.08</td>
</tr>
</tbody>
</table>
Table (4) demonstrates the concentration of Creatine Kinase (CK-MB) in the serum of the study groups. Different letters indicate a significant difference in the studied groups.

Despite the close association of obesity with heart and vascular diseases, and that the enzyme troponin is one of the regulatory enzymes present in heart tissues and is one of the main and most specific biomarkers for the early diagnosis of acute myocardial infarction and for assessing the degree of heart muscle damage in non-heart diseases that can negatively affect heart tissue cells (Chaulin., 2022), we do not find a noticeable increase in the concentration of the troponin enzyme. The increase was very slight and therefore there is no significant difference in our current study when comparing obese women with the control group since the obese group that was chosen for the study is healthy and that the troponin enzyme concentration increases during the first three hours since the onset of chest pain. However, in our current study, this slight increase in obese women may be a signal of the beginning. It is possible that the obese woman may suffer from one of the heart diseases in the future if obesity is not treated properly.

**Evaluation of Creatine Kinase CK-MB concentration in blood serum.**

The current study results, illustrated in figure (4), show a significant increase (P≤0.05) in the concentration of Creatine Kinase (CK-MB) in the blood serum of obese women (72.22 ± 56.83) UI/L compared to the control group (51.22 ± 17.6) UI/L. However, no significant difference was observed in the concentration of Creatine Kinase (CK-MB) in the blood serum of lean women (50.81 ± 27.08) UI/L compared to the control group (51.22 ± 17.6) UI/L.

Our current study agrees with Vasquez et al. (2020), whose results showed that CK increases with an increase in body fat mass, but not with an increase in muscle mass. The increase in body mass increases muscle stress and was a high indicator of CK release after fatigue. Their results also showed that resting CK levels were independently associated with body mass index in a diverse multi-ethnic group of patients. However, despite this association, baseline CK levels remained much lower than the upper normal limit when the body mass index decreased (Vasquez et al., 2020). Obesity is closely related to exercise intolerance and the development of heart failure in the person suffering from morbid obesity. The reaction rate of CK for heart muscle increases, maintaining ATP delivery despite the decrease in phosphocreatine / ATP during increased muscle stress. While the healthy heart of a person who has an ideal weight increases ATP delivery through CK, unlike the heart of an obese person, it does not. This is associated with a decrease in the contractile increase, exercise tolerance, and thus weight loss reflects these active changes. This sheds light on heart muscle energy delivery through CK as a potential therapeutic target to improve symptoms in obesity-related heart diseases (Rayner et al., 2020). In the study of Bekkelund et al. (2018), their study indicates that creatine kinase has an inhibitory effect on obesity-related inflammation via CK transfer or by other
muscle receptors. CK plays an important role in cellular energy metabolism in skeletal muscles. In addition, creatine enhances the respiratory rate in adipocyte mitochondria, thus increasing energy consumption and cellular heat. This suggests a possible mechanism for how CK interferes with obesity. There is also a connection between CK and inflammation. There can be several reasons for elevated CK. Previous studies that focused on conditions associated with hyperglycemia and inflammation have shown that an increase in creatine kinase CK can be explained by inflammatory muscle impairment in some cases. Inflammation may facilitate the release of CK into the bloodstream, especially during muscle effort. This increase in creatine kinase enzyme in our current study in obese women is the result of muscle stress due to obesity. Since CK is primarily derived from skeletal muscles and has a basic role in energy metabolism, its concentration increases in obese women.

**Conclusion**

The research indicates a strong correlation between obesity and a range of health risks, particularly those related to heart diseases. The biochemical markers studied, such as the adiponectin hormone, troponin I, and Creatine Kinase (CK-MB), have shown varying responses in obese and lean women, suggesting that body fat has a significant influence on these markers.

Notably, the concentration of the adiponectin hormone was found to decrease significantly in obese women, a trend which may contribute to various health complications including insulin resistance, type II diabetes, obesity, cardiovascular diseases, and fatty liver disease. On the contrary, the concentration of this hormone was found to increase in lean women, due to the energy balance maintained through the burning of fatty acids, indicating the protective role of adiponectin.

Although troponin I did not show significant difference among the studied groups, its slight increase in obese women could be an early indicator for potential heart-related diseases in the future, should obesity persist.

The study also observed a significant increase in the Creatine Kinase (CK-MB) concentration in obese women, potentially due to the muscular stress caused by obesity. However, no significant difference was found in lean women.

The study sheds light on the importance of maintaining a healthy weight to ensure the body’s optimal functioning and prevent serious health conditions. The findings underscore the necessity of implementing effective obesity management strategies, such as lifestyle changes and regular exercise, to lower the risks associated with obesity.

Further research is recommended to explore these findings in more detail, examining a broader array of biochemical markers in a larger and more diverse population sample, to better understand the complex interactions between obesity, metabolic health, and cardiovascular risk.

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