Ghrelin, resistin and insulin in obese diabetic women in Wad-Madani, Sudan

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Abstract

Background: Obesity in the Sudan is increasing at alarming rate with the tendency of reaching an epidemic proportion in women. It is commonly associated with type 2 diabetes (T2D). Some adipokine hormones such as resistin are associated with obesity.

Objectives: To study how the levels of resistin, ghrelin and insulin are associated with obesity, fat distribution and (T2D) and to ascertain any interrelationships between them.

Subjects and methods: 150 women, age ≥18 years old, resident in Wad-Madani town, Sudan were participated in the study. They were divided into 3 groups according to body mass index (BMI) value: I (normal weight), II (overweight) and III (obese diabetic). Fasting serum resistin and ghrelin concentrations were measured using ELISA method. Insulin levels were determined by radioimmunoassay (RIA).

Results: The mean±SD levels of resistin 5.80±4.91ng/mL, Ghrelin 107.60±26.67pg/M and Insulin 11.92±8.54mLU/ml in obese diabetic were found to be greater than in normal or overweight women. In normal weight values were 3.07±2.15 mg/mL 83.30±13.38pg/mL, and 6.62±6.77mLU/ml for resistin, ghrelin and Insulin, respectively. Values for overweight women 3.64±2.63 pg/mL 90±17.35 pg/mL and 8.13±7.54 mLU/ml for resistin, ghrelin and insulin respectively.

Conclusions and recommendations: Increased BMI, waist circumference (WC) and hormones (ghrelin and resistin) were associated with insulin resistance. Further studies are needed to accept or refute these findings.

Keywords: Obesity, BMI, WC, T2D, ghrelin, resistin and insulin resistance (IR).

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Introduction

Obesity is a community health problem accompanied by “metabolic syndrome,” which is stipulated to be affecting over one billion adults worldwide.¹ There is a reported dramatic rise in global obesity resulting in an increase in morbidity and mortality in humans, with morbid obesity being regarded as a real threat to health.² Metabolic complications of obesity in humans are associated with increased risks of various diseases such as insulin resistance, cardiovascular diseases, hypertension and certain type of cancers as earlier reported.¹ All of these have evolved as a result of unhealthy life styles. Generally, obesity is characterized by the excessive accumulation of fat in the adipose tissue which happens to be the source of several cytokines. The tissue act as an endocrine gland that shots hormones resistin into the blood to participate in their potency embodiment in Insulin resistance and diabetes.³ Inclusive of resistin, ghrelin and insulin also play vital roles in insulin resistance as they are facilitators in the pathogenesis of obesity. Researchers have demonstrated substantially that resistin provides the link between obesity, inflammation and atherosclerosis. Resistin belongs to a family of cysteine-rich proteins which have been linked to insulin resistance initially in rodents and then in humans.⁴ Resistin-like molecules are indicated to be presumptive adipocyte-derived signaling polypeptide generated in monocytes and macrophages associated with insulin resistance. Other studies postulate that resistin acts by decreasing insulin-stimulated glucose uptake.²

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Recent studies revealed that significantly increase in resistin levels were observed in obese patient than those in normal body mass subjects.³ Whereas, ghrelin, a gut hormone, is an appetite regulating hormone secreted during fasting by gastrointestinal endocrine cells and plays an important role in the coordination of energy balance and weight regulation.⁴ At the release of ghrelin, there is an activation of the growth hormone secretagogue receptor (GHS-R) within the anterior lobe of the pituitary gland, inducing the amount of food intake.⁵ unacylated ghrelin plays an important role in increasing fat tissue by reducing fat oxidation and encouraging the motility and gastric emptying. This process may imply a local effect and central mechanisms as well.⁶ His secretion levels are influenced by body fat and pubertal stage⁷ where they enhanced obesity and T2D in obese subjects.⁸ Moreover, the reduction occurs in ghrelin concentration may be related to the high caloric intake, whereas a reduction in body weight in obese patients caused elevation in ghrelin concentration level. This procedure could interpret the mark reduction of body weight in obese subjects.⁹ Grehlin was reported as one of the candidate genes responsible for obesity and T2D.¹⁰ Obesity and T2D in Sudan are increasing at alarming rate and have tendency to reach epidemic proportion in women. The objectives of this study was to estimate the levels of these hormones and their associations with Insulin resistance in obese diabetic women.

**Subjects and method**

**Ethical consideration**
All participants gave written consent for participation in the study and informed about the aim of the experiment. The study protocol was approved by the University of Gezira, Faculty of Medicine, to protect the rights and welfare of the human subjects (Sudan, acceptance letter No.0254/100/2012).

**Study subjects and experimental design**
A total of 150 women living in Wad-Madani, Sudan were used for the study. They were 18 years of age and above, divided into three groups based on their BMI classification. Anthropometric parameters such as age, height and weight were ascertained. WC was measured using cutoffs as recommended by WHO 2018.¹¹ Thus the women participants were divided into three groups according to the following: Low risk of disease ≤79 cm, increased risk of disease 80-87 cm and substantially increased risk of disease ≥ 88 cm. Body mass indices (BMI) were calculated according to BMI cutoffs proposed by the National Institutes of Health as follows: Underweight less than18.5, normal weight were between 18.5 to 24.9, 25.0-29.9 being overweight while 30.0 - 34.9 was obesity class one, and 35.0 - 39.9 Obesity class 2. Obesity class 3 were women with BMI ≥ 40.0.¹¹

Blood samples were collected for hormonal analysis from the volunteering women. We excluded pregnant women, age < 18 years old, chronic medical or psychiatric illness (with exception of obesity related diseases) and user of psychiatric drugs.

**Biological material preparation and determination of hormonal plasma concentration**
A trained nurse assisted with blood sample collection. After overnight fasting, blood samples from them were collected and put in heparinized test tubes. Plasma was separated by centrifugation at (1000g, 15 min.) after which samples were divided into several portions and stored at -70°C for future use. Resistin and ghrelin levels were determined using ELISA. The hormonal concentrations in plasma were determined by immunoenzymatic methods using Human Quantikine ELISA Kit (R&DSystems,USA) according to the manufacturer's guidelines. The insulin level was determined by radioimmunoassay (RIA). This technique, using (IMK-414, China) kit according to the manufacturer's guidelines. The standards of the kit have insulin values ranging from 5 to 160mlU/L.

**Statistical analysis**
Data was recorded and analyzed using SPSS version 16.0. Independent samples t-test and pearson correlation coefficient were used to determine significance between relationships and comparison of the hormone levels. Differences among groups were compared by using one way ANOVA and least significant differences (LSD) for comparing means among hormonal levels, BMI and WC.

**Results**
The present study revealed a highly positive significant correlation between the (BMI) and the WC of the participants. Table: 1 illustrates the mean of BMI, age and WC of the study groups.
Table 1: Mean BMI, age and WC of the three study groups

| Group number | Mean BMI ± SD | Mean age ±SD | Mean WC ±SD |
|--------------|---------------|--------------|-------------|
| Normal weight | 22.01±2.09 | 36.68±13.50 | 90.46±6.40 |
| Overweight | 27.26±1.96 | 40.12±11.31 | 104.04±9.11 |
| Obese diabetic | 37.95±10.21 | 53.52±12.55 | 110.5±11.19 |

Table 2: Shows differences among hormonal levels, BMI and WC in study participants by using one way ANOVA test

| Source of variations | Sum of square (SS) | Square (df) | Means of squares (MS) | Calculated F-value | Critical F-value | P value |
|----------------------|--------------------|-------------|-----------------------|--------------------|------------------|---------|
| Between groups       | 53.25              | 2           | 26.6                  | 30.2               | 2.996            | p=0.05* |
| Within groups        | 129.9              | 147         | 0.88                  |                    |                  |         |
| Total hormones       | 183.15             | 149         |                       |                    |                  |         |

| Source of variations | Sum of square (SS) | Square (df) | Means of squares (MS) | Calculated F-value | Critical F-value | P value |
|----------------------|--------------------|-------------|-----------------------|--------------------|------------------|---------|
| Between groups       | 136                | 2           | 68                    | 75.6               | 2.996            | p=0.05* |
| Within groups        | 133.4              | 147         | 0.90                  |                    |                  |         |
| Total WC             | 269.4              | 149         |                       |                    |                  |         |

| Source of variations | Sum of square (SS) | Square (df) | Means of squares (MS) | Calculated F-value | Critical F-value | P value |
|----------------------|--------------------|-------------|-----------------------|--------------------|------------------|---------|
| Between groups       | 60.63              | 2           | 30.315                | 43.3               | 2.996            | p=0.05* |
| Within groups        | 102.52             | 147         | 0.7                   |                    |                  |         |
| Total BMI            | 163.15             | 149         |                       |                    |                  |         |

Table 2 demonstrates differences among hormonal levels, BMI and WC in study participants by using one way ANOVA test and LSD for comparing means between and within groups.
Whereas, Table (3) demonstrates the comparison between the mean levels of ghrelin, resistin and insulin in normal weight, overweight and obese diabetic. Differences among means level of hormones across groups were compared as shown in Table: 2

Table 4: Correlations of the hormones with the BMI and WC of the study groups.

| Groups               | parameters | correlations | Insulin | ghrelin | Resistin |
|----------------------|------------|--------------|---------|---------|----------|
| Normal weight        | BMI        | R .067       | -.137   | .201    |          |
|                      | P          | .644         | .020    | .162    |          |
|                      | WC         | R -.063      | .034    | .034    |          |
|                      |            | P .665       | .813    | .813    |          |
| Overweight           | BMI        | R .059       | .124    | -.039   |          |
|                      | P          | .685         | .391    | .786    |          |
|                      | WC         | R -.209      | .083    | -.041   |          |
|                      |            | P .146       | .565    | .777    |          |
| Obese diabetes type 2| BMI        | R -.072      | .047    | -.097   |          |
|                      | P          | .621         | .748    | .502    |          |
|                      | WC         | R -.016      | .022    | .022    |          |
|                      |            | P .910       | .881    | .881    |          |
However, Table (4) shows the correlations of the measured hormones with the BMI and WC of the study groups. Significant difference (P ≥0.05) were registered among insulin, resistin and ghrelin in the different groups. Contrary, both resistin and insulin were insignificant in overweight and normal weight when compared within group. There are significant correlations registered for resistin and ghrelin in overweight group when compared across different study groups.

Examining, WC in normal weight and overweight women revealed that, there is overlap of the scatter of insulin values and there is no significant difference between them (p = 0.297). This indicates that WC does not play a role in these groups. The correlation between the insulin hormone and WC in overweight and obese diabetic women revealed that, the hormone has significant difference when the two groups were compared (p=.020).

| Table: 5 Correlation Samples of studied hormone in different study subjects |
|-----------------------------------------------|
| Body weight      | Pair            | r    | P    |
| Normal weight    | Insulin Vs Grehlin | .091 | .528 |
|                  | Insulin Vs Resistin | .125 | .388 |
|                  | Grehlin Vs Resistin | -.001 | .996 |
| Overweight       | Insulin Vs Grehlin | -.224 | .117 |
|                  | Insulin Vs Resistin | -.278 | .051 |
|                  | Grehlin Vs Resistin | -.085 | .556 |
| Obese Diabetic   | Insulin Vs Grehlin | .025 | .865 |
|                  | Insulin Vs Resistin | -.017 | .904 |
|                  | Grehlin Vs Resistin | .098 | .498 |

Table (5) shows the correlation coefficient among the studied hormones when we studied each versus another in all groups. In the present study six pairs of the hormonal correlations were obtained. No significant correlation, were registered for any pairs versus each other in normal weight overweight and obese diabetic subjects. Nevertheless, the mean insulin levels in the overweight is slightly higher than in normal weight subjects but the difference is insignificant, and still within the normal non-diabetic range. However, the mean insulin level is significantly higher in obese diabetic subjects when compared with both overweight and lean control subjects. Fig. 1 (A and C) shows the correlation between the insulin, BMI and WC in normal weight and overweight women whereas, Fig. 1 (B and D), illustrate the correlation between insulin, BMI and WC in overweight and obese diabetic groups.
Fig.1: The distribution between Insulin hormone, BMI and WC among the study group. A: Correlation between the Insulin hormone and BMI in normal weight and overweight. B: Correlation between the Insulin hormone and BMI in overweight and obese diabetic. C: Correlation between the Insulin hormone and WC in normal weight and overweight. D: Correlation between the Insulin hormone and WC in overweight and obese diabetic.
Fig. 2: The distribution between Grehlin hormone, BMI and WC among the study group. A: Correlation between the Grehlin hormone and BMI in normal weight and overweight. B: Correlation between the Grehlin hormone and BMI in overweight and obese diabetic. C: Correlation between the Grehlin hormone and WC in normal weight and overweight. D: Correlation between the Grehlin hormone and WC in overweight and obese diabetic.
Figures (1; 2 and 3) illustrate the correlations between the means level of measured hormones with BMI and WC of the study groups. The mean of hormonal levels of insulin, resistin and ghrelin increase with increasing BMI and WC values for the study participants.

Discussion

The present study indicated that the level of insulin, resistin and ghrelin are higher in obese T2D subjects when compared with lean and overweight subjects. These results correlate positively with obesity and mark an important link between obesity and related metabolic syndrome.

Nonetheless, the main findings of the current study verified significant positive correlation between BMI and insulin in overweight and obese T2D women. In addition, insulin levels were significantly high in obese diabetic group, which confirmed that obesity in women is associated with hyperinsulinaemia and insulin resistance. These results are explained by the close relation between BMI WC and body fat content or by the overall significance of abdominal fat. These findings might likely refer basically to several mechanisms that could verify or show how visceral adiposity in obese subjects, could lead to insulin resistance IR. On the other hand, fat deposits areas, mainly visceral fat, released free fatty acids (FFA). These acids can block the insulin signal pathways directly and intersected insulin secretion as well as insulin action. Moreover, increased amounts of FFA in the portal circulation may impair the metabolism and action of Insulin and directly increase gluconeogenesis in the liver. The correlation coefficient between the insulin and WC in normal weight and overweight women demonstrated that, there is overlap of the scatter of insulin values and exhibited no significant difference between the two groups, (p=0.297). The correlation between the
insulin and WC in overweight and obese (T2D) women has shown that, the hormone has significant difference when the two groups were compared (p=.020). These findings are in accordance with other studies showing elevated serum insulin levels in obese subjects.\textsuperscript{1,12} Similar findings were established in Egypt\textsuperscript{14} and in Saudi women.\textsuperscript{15} Correlation between resistin and BMI revealed that resistin level is slightly higher in obese T2D women when compared with both over-weight and lean subjects. The consequences might refer to the low levels of of adipose tissue in overweight when compared to the obese diabetic participants. Therefore, overweight subjects do not show a significant rise in resistin compared to normal individuals.

However, obese diabetic subjects show significant rise in resistin which is in agreement with the insulin resistance in T2D patients. These results are consistent with the studies completed in Saudi women and others in Jordan who reported higher resistin levels were observed in patients with T2D and were correlated with insulin resistance and obesity proposing that resistin might contribute to the development of T2D in humans.\textsuperscript{15} Later, several recent researches in humans have shown that higher serum resistin in obese patients was linked to obesity concentrations when compared with lean subjects. This outcome was found to be positively associated with visceral fat area and changes occurring in BMI.\textsuperscript{16} Contrasting studies in Caucasian and non-Caucasians populations show divergent outcome suggesting the role for resistin in obesity.\textsuperscript{17} Nonetheless, different studies have supported increased resistin expression with adiposity and others have shown a significant decrease in revolving resistin concentrations. These findings were noticed to be found accompanied with the moderate weight loss and post-gastric bypass. No plasma levels of resistin with markers of adiposity was reported.\textsuperscript{18} However, the present data regarding resistin adds to a growing body of evidence that resistin is strongly expressed in human visceral fat chambers and is more highly affected by WC and BMI. Thereupon, these results recommended that WC is an adequate rightious index for visceral fat cumulation and can therefore, be used as a sensitive indicator of health hazards correlated with visceral obesity. This outcome also confirms that resistin is an important hormone in human adipose tissue. Our results go in the same trends with Guo.\textsuperscript{19}

The current study showed that ghrelin levels scored higher concentrations in obese subjects than in health bodies. The results go in the same line of the studies conducted in human by Al-Hakeim.\textsuperscript{20} Similar findings were found in Caucasian and Pima Indian individuals\textsuperscript{21,Egypt} and in Saudi females.\textsuperscript{15} Regarding our data higher ghrelin levels were recorded in obese subjects when compared with the lean and overweight. This may be either due to obesity and its complications, or partly due to the active feedback that enhances appetite and body weight, but not the main cause of obesity. This could be supported by the fact that circulating ghrelin levels increased in anorexia nervosa, syndrome and cachexia.\textsuperscript{22} These data seem to elucidate that ghrelin served as a regulatory tool in human obesity. Thereupon, this tactic may disprove the result of elevated insulin. This could be attributed to, the levels of fasting plasma ghrelin which are positively correlated with insulin fasting plasma levels.

Therefore, the adequacy of plasma ghrelin concentrations resulted in initiating obesity and this may also imply a physiological adaptation to the positive energy balance that correlated with obesity occurrence. Further information is critically needed to probe the exact effect of ghrelin and why ghrelin sufficiency occurs in obese individuals. Several studies have asserted that the mechanism of obesity is still unclear. Uncontrolled diabetes leads to increase both revolving ghrelin concentrations and behavioral sensitivity to ghrelin. Although plasma ghrelin concentrations fall in response to hyperphagic feeding. These outcomes support the hypothesis that high lighted increased ghrelin signaling contributes to the pathogenesis of diabetic hyperphagia.\textsuperscript{23} Despite using the Pearson correlation test, it is worthy noting that there is no significant correlation among the serum concentrations of studied hormones in all study groups specifically obese with T2D subjects. These results indicate very weak inter-relationship among them. No interrelationship between hormones could be explained by the small number of patients involved in the study. Interestingly, the relationship of insulin, ghrelin and resistin in normal, over weight and obese T2D women have been reported for the first time in Sudan and our findings could be indorsed as the baseline data for future studies and population health care.

**Conflict of interest disclosure**
All authors declare that no conflict of interest exists for this work.
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