Relationship Between Leptin and Thyroid Hormones In Type 2 Diabetes Mellitus Patients

Ghena Hamoudi Hussein Alzubaidy  
Talib Abdul Hussein Mousa  
AL-Muthanna University -College of Science  
Abdul Hussein Mahdi Aljibouri  
Babylon University- College of pharmacy  
abduhhussien@hotmail.com

Abstract:
This study was performed in the Diabetes and Endocrine Center at Al-Husain Teaching Hospital. The patients and control groups were with age ranged between (40-83) years. Study was carried out on 60 patient with type 2 diabetes mellitus (28 male and 32 female) and 20 apparently healthy subjects male and 20 female how dealt with as control group. Leptin and thyroid hormones(T3,T4, AND TSH) measurements were carried out using ELISA technique. Both patient and control groups were classified according to obesity, age, and gender. Results of the present study show that leptin hormone was significantly high in serum of type 2 diabetic patients group compared with control group (P < 0.001).also there was a significant gender difference (female more than male) and significant positive correlation with body mass index and body fat. Results also show that TSH hormone and FBS were significantly high(P < 0.000), while T3and T4 decrease significant(P < 0.000) in type 2 diabetic patients group and compared with control group.

Keywords: type 2 diabetes mellitus, Leptin , thyroid hormones , lipid profile

Introduction
Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Two major forms of diabetes were identified; type 1 and type 2. Lack of or severe reduction in insulin secretion due to autoimmune or viral destructions of β-cells is responsible for type 1 diabetes (ADA, 2011), which accounts for 5-10% of diabetic patients(Olefsky, 2001). The more prevalent form, type 2 diabetes, accounts for more than 90% of cases. Type 2 diabetes usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to reduce it (Kasuga, 2006).

Lack of insulin action and/or secretion in type 2 diabetes induces hepatic glucose output by inhibiting glycogen synthesis and stimulating glycogenolysis and gluconeogenesis then increased rates of hepatic glucose production result in the development of overt hyperglycemia, especially fasting hyperglycemia (Defronzo and Simonson, 1992; Michael et al., 2000). In such conditions,
lipolysis in adipose tissue is promoted leading to elevated circulating levels of free fatty acids. Ketone bodies are produced, and are found in large quantities in ketosis, the liver converts fat into fatty acids and ketone bodies which can be used by the body for energy (Botion and Green, 1999). In addition, excess fatty acids in serum of diabetics are converted into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins (Jaworski et al., 2007).

Leptin is a small peptide hormone (16-kDa protein) that is mainly, but not exclusively, produced in adipose tissue. The circulating leptin concentration therefore directly reflects the amount of body fat (Merabet et al., 1997; Wolf et al., 2001).

Leptin was identified through positional cloning of the obese gene, which is mutated in the massively obese ob/ob mouse, and it has a pivotal role in regulating food intake and energy expenditure (Zhang et al., 1994). On the light of the present results, negative significant correlation was recorded between serum glucose and leptin. There is many evidences supporting the statement that changes in insulin secretion and glucose metabolism are the major mediators of leptin production by adipose tissue (Havel, 2000). Insulin level can stimulate the transcriptional activity of the leptin promoter (Moreno-Allaga et al., 2001). Leptin is a protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. It is one of the most important adipose derived hormones (Brennan and Mantzoros, 2006).

Leptin concentration was found to widely vary in between men and women, but in women was much higher than in men more specifically. Mean concentration in women is 12.7 mg/L while it is 4.6 mg/L in men, leptin itself was discovered in 1994 by Jeffrey M. Friedman and colleagues at the Rockefeller University through the study of such mice (Efstratiads et al., 2007). Leptin acting on its receptors in hypothalamus and other several peripheral tissues exerts diverse biological effects including regulation of energy homeostasis, glucose metabolism, lipid oxidation, reproduction, blood pressure, hematopoiesis, angiogenesis, brain and bone development, wound healing, and cell differentiation and proliferation (Fantuzzi and Faggioni, 2000). Also the hypothalamic-thyroidal axis is regulated by leptin. This provides an important interface between adiposity, regulated by leptin and metabolic rate, regulated by thyroid hormone (Kim et al., 2000). Thyroid hormones play a very important role in controlling the body's metabolism, that is, the rate at which the body uses energy (Stipanuk, 2000), by stimulating divers metabolic activates most tissue, leading to an increase in basal metabolic rate one consequence of this activity is to increase body heat production (Choksi et al., 2003). So thyroid hormones with leptin might be involved in the adaptive thermo genesis (Zimmermann-Belsing et al., 2003). The present study is intended to asses and study correlation between serum levels of leptin and Thyroid hormones and TSH in type 2 diabetic patients in both males and females.

Materials & Methods:

The study included (80) subjects (38males and 42females), 40 healthy subjects as control group, and 60 patients of type 2 diabetic mellitus (28males and 32females) attended to the diabetic and endocrinology center at Al-Hussein medical hospital in Al-Muthana. Patients with hepatitis, renal failure, liver disease, malignant disease, patients on chemotherapy, patients with complications of diabetes like diabetic neuropathy, nephropathy, retinopathy, or chronic inflammatory diseases, based on clinical and laboratory investigations. Also smokers and alcoholic patients all of these types were excluded.
Questionnaire of each patient was taken, it included: age, duration of diabetes mellitus, types of antidiabetic treatment, family history of diabetes, weight and height.

Blood samples were collected between 8:30-10:00 A.M. after 12 hour fasting from Patients and control groups. The samples were taken five milliliters of blood was obtained by antecubital venipuncture using G23 needle, the remaining blood was allowed to clot in test tube at room temperature, the serum was aspirated after centrifugation at 2500 cycles / minute for 15 minutes, divided into aliquots in epindroff tubes and stored at -20°C until measure of hormones(T3, T4, TSH, and Leptin), but measurement directly after the collection the fasting blood sugar (FBS)

The following laboratory investigations were done for all patients and control subjects:
1-Serum Leptin levels were measured in duplicate by radioimmunoassay (Leptin ELISA Kit. Diagnostic System Laboratories (DSL)/USA)
2-Serum levels Thyroid Hormones were measured in duplicate by radioimmunoassay (Thyroid Hormones ELISA Kit (T3, T3 and TSH). Immunotech a.s/France)
3-Serum fasting blood levels were measured in duplicate by radioimmunoassay (Glucose kit. Diasys Diagnostic Systems, Germany)

Statistical Evaluation Methods:

Data were analyzed using SPSS(Version 15) the significance of difference was tested using spss analysis and statistical test were performed using a null hypothesis of no difference student t-test. The level of significance and P-value were <0.05 and <0.01.

Results and Discussion

Diabetes is a family of disorders that is characterized by hyperglycemia (Wendy, 2007). Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia factors contributing to hyperglycemia may include reduced insulin secretion (Dennis et al., 2005).

Relationships between Leptin and thyroid hormones:

The results in table (3 – 1) showed a significant increase (P≤ 0.001) in level of Leptin hormone, while there were significantly high (P≤ 0.000) in levels of thyroid-stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4), hormones in males and females patients with diabetes mellitus compared with control group.

Table (3-1): Serum level of Leptin and Thyroid hormones components in patients and control groups according to gender, age and BMI.
### Groups

<table>
<thead>
<tr>
<th>Leptin (ng/ml)</th>
<th>Control n=40</th>
<th>Patients n=60</th>
<th>B.M.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>0.225</td>
<td>0.31</td>
<td>3.2±1.072</td>
<td>2.658±1.278</td>
</tr>
<tr>
<td>±0.132</td>
<td>±0.226</td>
<td>±0.132</td>
<td>±0.226</td>
</tr>
<tr>
<td>2.658±1.278</td>
<td>4.77±2.232</td>
<td>2.445±0.427</td>
<td>4.65±2.067</td>
</tr>
<tr>
<td>4.063±0.921</td>
<td>5.438±2.268</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>T3 (nmol/l)</td>
<td>4.36</td>
<td>4.6</td>
<td>2.5</td>
</tr>
<tr>
<td>±2.542</td>
<td>±2.641</td>
<td>±1.135</td>
<td>±0.901</td>
</tr>
<tr>
<td>2.175±0.183</td>
<td>1.775±0.661</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>T4 (nmol/l)</td>
<td>6.56</td>
<td>9.35</td>
<td>4.275±1.191</td>
</tr>
<tr>
<td>±1.892</td>
<td>±5.51</td>
<td>±1.191</td>
<td>±1.067</td>
</tr>
<tr>
<td>11.078±2.054</td>
<td>11.729±2.405</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4.309±1.063</td>
<td>4.714±2.093</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4.15±1.934</td>
<td>5.1±0.721</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>FBS (mmol/L)</td>
<td>6±1.558</td>
<td>5.777</td>
<td>19.313±5.192</td>
</tr>
<tr>
<td>±1.335</td>
<td>±1.335</td>
<td>±5.192</td>
<td>±4.669</td>
</tr>
<tr>
<td>19.727±5.517</td>
<td>19.421±4.66</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>20.563±4.259</td>
<td>20.613±4.63</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

- All results show significant difference at (P<0.05)
- G1=age(40-59 year), G2=age(60-83 year)
- 1=normal weight, 2=over weight, 3=obese

Table (3-2): Correlation between serum leptin and Thyroid hormones in patients and control groups(r values)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control n=40</th>
<th>P value</th>
<th>Patients n=60</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3 (nmol/l)</td>
<td>-0.724</td>
<td>-0.1</td>
<td>-0.777</td>
<td>-0.4</td>
</tr>
<tr>
<td>T4 (nmol/l)</td>
<td>-0.931</td>
<td>-0.1</td>
<td>-0.978</td>
<td>-0.4</td>
</tr>
<tr>
<td>TSH(mIU/ml)</td>
<td>-0.58</td>
<td>-0.1</td>
<td>-0.877</td>
<td>-0.1</td>
</tr>
<tr>
<td>FBS(mmol/L)</td>
<td>-0.59</td>
<td>-0.1</td>
<td>-0.5</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

Firstly: In this study found that the leptin hormone concentration increase in patients with diabetes mellitus compared with control group, and there is a significant increase (P≤0.052) in female more than male in diabetic patients. Also the study revealed a significant increase leptin
levels with increase BMI at (P≤ 0.026) compared with normal weight group , (P≤ 0.047) of overweight and (P≤ 0.000) of obese and different age at (P≤ 0.000) of group one (40-59 years) and (P≤ 0.054) of group two (60-83 years).

The results of the present study are in good agreement with previous studies regarding the role of leptin in diabetes mellitus patients (Al-Daghri et al., 2003), nevertheless other reports indicate decreased (Sivitz et al., 2003; Marita et al.,2005) or unchanged serum leptin levels in diabetes mellitus patients (Haffner et al.,1996 ; Snehalatha et al.,1999).

Leptin a hormone known mainly for regulating appetite control and energy metabolism, plays a major role in islet cell growth and insulin secretion Part of the controversy among previous reports could be related to the differences in adiposity or gender of the patients (Wauters et al., 2003). Leptin concentration is modulated by weight gain and loss in adult humans. Obesity is strongly associated with the development of diabetes mellitus. Adipose-derived hormone, leptin has been implicated in the regulation of body weight and energy homeostasis. Circulating leptin concentrations reflect the amount of adipose tissue in the body (Caro et al.,1996; Van Gaal et al.,1999). Strong relationship between diabetes mellitus and obesity so the elevated leptin might be due to increased body mass Serum leptin increases with increasing body fat mass (Considine et al.,1996; Leonhardt et al.,1998; Lissner et al.,1999; Ruhl et al.,2001; and Baig et al.,2003). Leptin showed significant positive correlation with BMI. Similar result was declared (Mohamed Ali et al., 1997; Nakazona et al., 1998; Nevalainen et al.,2000; Al-Holi, 2006; and Marjani et al., 2010). Serum leptin concentration is increased in obese subjects and is closely related to fat mass and BMI, it is regulated by serum insulin concentration and declines with weight loss (Rosenbaum et al.,1996; Woods et al., 2003). Circulating leptin levels appears to be one of the best biological markers of obesity and hyperleptinemia is closely associated with several risk factors related to obesity syndrome (Mohiti and Qujuq ,2005).

Increase in the content of fat depot is reflected by increase in the adiposity signal; leptin, even in lean subjects (Cnop et al., 2002). The inability of such elevated leptin levels in most obese individuals to alter the obese state of subjects may be related to "leptin resistance " (Gonzalez et al.,2000), an inability of leptin to enter the cerebral spinal fluid to reach the hypothalamic regions that regulate appetite, or it may simply reflect the large amount of fat tissue in the body (Emanuelli et al., 2001). Most obese individuals are leptin-resistant (Widjaja et al., 1997), resistance to the actions of leptin could be cause by decreased leptin transport through the blood-brain barrier (Ur E et al.,1996; Schawatz et al.,1996) or to reduced signaling distal to the leptin receptor (Ur E et al.,1996; Frilhebecu and Salvador,2000). Peripheral signals such as glucocorticoids may also interfere with leptin’s interaction with its receptor and produce central leptin resistance (Rink,1994; Frilhebecu and Salvador, 2000). Women tendency to have a higher overall obesity which is more pronounced in subcutaneous fat than in visceral fat, in contrast to men who have a lower overall but greater visceral adiposity (Hadjji et al., 2000). The higher leptin levels in females than in males have previously been reported in population studies (Lonnqvist et al., 1995; Wabitsch et al.,1996; Panarotto et al.,2000; Hadji et al.,2000) and are probably due to gender differences in body fat distribution. Subcutaneous fat produces more leptin than visceral fat(Montague and O'Rahilly,2000; Cnop et al.,2002) . Women, carry most of their higher body fat content subcutaneously, whereas men carry most of their lower body fat content viscerally(Van et al.,1999; Montague and O'Rahilly,2000; Blaak et al.,2001; Mendoza et al., 2002; Woods et al., 2003). The higher subcutaneous fat content in women would, therefore, explain their higher serum leptin concentrations. It however, has also been suggested that the gender differences in serum leptin may be related to the differences in sex.
hormones (Van et al., 1999). This result supported by other study that demonstrated an independent effect of age on serum leptin concentration (Ostlund et al., 1996). Similar results were found by (Perry et al., 1997; Andrea et al., 2000; Marita et al., 2005; Al-Holi, 2006).

Secondly: For thyroid hormones the results show an increase in TSH level while there were decrease in both T3 and T4 levels in patients with diabetes mellitus compared with control group and without significant differences between female and male in diabetic patients. The study also showed the existence of significant difference between thyroid hormone and BMI and age. In case of BMI for those with normal weight difference be moral (P ≤ 0.001), for T3, (P ≤ 0.008) for T4, and (P ≤ 0.000) for TSH respectively, as for the owners of the excess weight the difference be moral (P ≤ 0.000) for all thyroid hormones , While obesity owners be moral differences have (P ≤ 0.000) for T3andTSH,( P ≤ 0.005) for T4. When age was take in consider age difference be moral for the first group (P ≤ 0.000) for all thyroid hormones, as for the second group (P ≤ 0.000) for T3and TSH,( P ≤ 0.003) for T4 in diabetic patients compared with control group this study agrees with (Rahbani et al., 2004; Yu et al., 2006; Kale et al., 2007).

The inter-relationship between obesity and thyroid hormones in determining that the circulating levels of leptin is still a matter of discussion, although the major determinates of leptin levels, are gender and amount of fat, other factors are likely to be implicated in determining circulating leptin concentrations, thyroid hormones exert a negative effect on leptin secretion in vitro (Pinkney et al., 1998; Leonhardt et al., 1998; Tagliaferri et al., 2001). As far as thyroid function in concerned, hypothyroid patients had been reported to have higher levels of leptin than healthy subject matched for body mass index (BMI) (Leonhardt et al., 1998).

Thyroid hormones which produce over activity of sympathetic nervous system, resulting in the increase release of norepinephrine from sympathetic nerve endings in adipose tissue, the fat cells express adrenergic receptors that are stimulated by norepinephrine, causing fatty acid hydrolysis and also uncouple energy production from fat store of hyperthyroid patients, low serum leptin level in hyperthyroid patients is due to hyperadrenergic state found in these patients and /or it may be the result of suppression of leptin gene expression due to overactivity of TSH receptors by auto antibodies (Baig et al., 2003). As both thyroid hormones and leptin have effects on similar aspects of body homeostasis, the cross-talk between thyrostat and the lipostate might play a crucial role in the maintenance of the body homeostasis. Thyroid hormones were shown in vitro to increase leptin messenger ribonucleic acid (mRNA) expression and secretion in fully differentiated adipocytes (Ghizzoni et al., 2001).

Leptin and TSH might indicate a positive regulation of hypothalamus pituitary thyroid axis by leptin through its direct interaction on TRH-Synthesizing neurons in the paraventricular nucleus and/or indirectly through the POMC/ agouti- related peptide pathway (Flier et al. 2000; Ghizzoni et al., 2001; Kim et al., 2001). Thyroid hormones have a permissive role on the effects of catecholamines on B-adrenergic receptors, astimulation of these receptors suppresses leptin expression, thyroid hormones might exert an inhibitory effect on leptin secretion through activation of these receptors (Kopp et al., 1997; Ghizzoni et al., 2001).

Thyroid insufficiency leads to generalized slowing of calorigenic metabolism, including over all decreases in daily energy intake, oxygen consumption, and thermogenesis (scarpace et al., 1997; Bornstein et al., 1997).

Thirdly: For fasting blood glucose level increase in patients with diabetes mellitus compared with control group in both females and males, and without significant differences between females and males in patients groups. The study also showed the existence of significant difference between insulin hormone and BMI and age for the BMI for those difference be moral
(P ≤ 0.000) with all group of BMI, as for the age difference is moral for the first group (P ≤ 0.000) for all group of age in diabetic patients compared with control group. These results are expected due to the fact that the main characteristic feature of DM is hyperglycemia. Blood glucose is tightly controlled by two key processes:- insulin secretion by pancreatic β-cells in response to a nutrient and insulin action on major target organs, i.e., skeletal muscle, liver, and adipose tissue. T2DM, is often associated with obesity and results from insufficient insulin production/secretion and IR (Paz et al. 2006). There is much evidence supporting the statement that changes in insulin secretion and glucose metabolism are the major mediators of leptin production by adipose tissue (Havel 2000). The endogenous glucose production decreased significantly during leptin treatment, an effect that can be attributed primarily to a reduction in glycogenolysis, leptin treatment improved the ability of insulin to suppress endogenous glucose production, with significant decreases in both glycogenolysis and gluconeogenesis (Kathleen et al. 2009). The metabolic effects of leptin participate in the regulation of hepatic glucose metabolism under physiological conditions (Abdelgadir et al. 2002). May be disorder in pancreas to lead decreased secreted insulin hormone or sensitivity target tissue of insulin to lead increase of the level of blood sugar (Arthur and John, 2006; Dennis et al., 2005; and Wendy and Jean, 2007).

Conclusions:-
1. Type 2 DM was found to be associated with hyperleptinemia in both gender.
2. Leptin level was higher significantly in obese and overweight diabetic patients.
3. Leptin level was higher significantly in females than males, but not significant change in different age stage.
4. TSH level was higher significantly in diabetic patients, but T3 and T4 level were lower significantly in diabetic patients.

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