Effect of experimentally induced diabetes mellitus on serum leptin level and the role of insulin replacement therapy

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Abstract

The regulation of circulating leptin concentration is multifactorial and still understood. Therefore, the present study was designed: (1) to demonstrate the effect of gender on leptin concentration in intact control rats, (2) to evaluate the relationship between serum leptin concentration, serum insulin level and body weight in control rats and streptozotocin (STZ) diabetic rats (untreated and treated with insulin). A total number of 48 healthy adult albino rats of both sexes (24 males & 24 females) were used in this study. The animals were divided equally into four groups, each group was subdivided equally into male and female subgroups. Group 1(G1): served as control group, group 2(G2): experimental diabetics group (not treated) and group 3(G3) & group 4(G4): experimental diabetic group treated with insulin for 2 and 21 days. These groups were examined for the following parameters: body weight, blood glucose level, serum insulin and leptin concentration. It was found that serum leptin level was significantly higher in female than in male control group. However, this difference could not be detected in between male and female rats in all other studied groups. Moreover, a strong positive correlation was found between leptin concentration and insulin hormone level, and body weight in control male and female rats. STZ-induced diabetes associated with a significant rapid decrease in circulating leptin concentration (G2), this decrease was accompanied with a significant decrease in both serum insulin level and body weight, then, it was rapidly reversed by insulin treatment for 2 days (G3) and 21 days (G4). There was also a positive correlation between serum leptin concentration, body weight and serum insulin level in this diabetic group. However, leptin was found to change in an inverse proportion to the variation in blood glucose concentration in both male and female diabetic groups. The direct relationship of serum leptin concentration to serum insulin concentration and the inverse relationship of leptin concentration to blood glucose level were preserved in both male and female insulin replacement therapy groups.

In conclusion, in adult albino rats, several factors are involved in the regulation of circulating leptin level such as gender, body weight, blood glucose level and serum insulin concentration.

Introduction

The discovery of the obese gene in the mouse and its conserved homologue in humans has led many researchers to work hardly to find out the factors which could regulate the expression of leptin gene and in turn could regulate metabolism and behavior (Saladine et al., 1996). Leptin was first described as an adiposity derived signaling factor, which, after interaction with specific receptors, induces a pleiotropic response including control of body weight and energy expenditure. Although research has moved ahead rapidly there

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are still more questions about the regulatory pathways that control leptin and modulate leptin expression (Auwerx and Stales, 1998).

Several lines of evidence suggested that the hypothalamus is a critical target for the satiety effect of leptin. This effect may be mediated partly by inhibition of neuropeptide Y (NPY) which is a potent stimulator of food intake (Erickson et al., 1996). Other candidates of which activity could be modulated by leptin and which are active in the brain are melanocyte stimulating hormone (Huszer et al., 1997), glucagon-like peptide, corticotropin releasing hormone or related factor urocortin), and melanin concentrating hormone (Spina et al., 1996).

A remarkable gender difference in serum leptin levels in both human and animal studies were reported. It was found that intact female rats had a higher serum leptin level than intact male rats (Yacout et al., 2000). Moreover, profound gender differences in circulating leptin was documented in adult humans (Havel et al., 1996), children (Lahlou et al., 1997), and healthy subjects and young patients with insulin dependent diabetes mellitus (IDDM) (Verroti et al., 1998).

Previous studies reported that the amount of leptin mRNA in adipocytes correlates with body weight (Hamilton et al., 1995). It was found that circulating leptin concentration is elevated in the obese state; decrease following weight loss and increase again with weight (Nicklas et al., 1997). Moreover, it was reported by Considine et al. (1996) that a reduction of 10% in body weight is associated with 53% reduction in serum leptin. Even a 4% reduction in body weight over a period of 7 days resulted in 61% decrease in leptin values in both sexes (Dubuc et al., 1998). In addition, Caro et al. (1996) reported that a small (10%) increase in body weight results in a 300% increase in serum leptin.

A strong positive correlation was reported between serum leptin concentration and body fat content and serum insulin level (McGreger et al., 1996). In addition, some but not all investigators found that insulin is a potent regulator of plasma leptin level regardless of their relation-ship with body weight and total body fat (Ahren et al., 1997 and Kiess et al., 1998). However, other investigators suggested that insulin does not stimulate leptin production (Dagog-Jack et al., 1996). Regulation of (ob) gene expression by insulin is also supported by findings that (ob) gene expression is reduced in insulin dependent diabetes which is accompanied by hypoinsulinemia (Sivitz et al., 1996 and Sivitz et al., 1998). However, Tuominen et al. (1997) demonstrated that leptin levels were higher in insulin dependent diabetes mellitus. In addition, Verroti et al. (1998) showed that type I diabetes does not modify serum leptin concentration.

Some studies showed that insulin therapy resulted in normalization of lower leptin levels in diabetic animals (Mac Dougard et al., 1998), while others did not (Backer et al., 1995).

Because of the variability of information mentioned and the contradiction of reports about some of the factors that could regulate the serum leptin levels, this study was designed to:

1. Demonstrate the effect of gender on leptin concentration in the intact control group and evaluate the effect of gender under the influence of some metabolic disorders as experimentally-induced diabetes mellitus (untreated and treated with insulin).

2. Evaluate the relationship between leptin concentration, serum insulin level and body weight in control
intact rats, experimentally diabetic rats (untreated and treated with insulin).

3. Study the effect of experimental diabetes and Insulin replacement therapy for 2 days and for 15 days on serum leptin concentration.

**Material & Methods**

**Experimental Animals:**
A total number of 48 healthy adult albino rats of both sexes (24 adult males & 24 adult females) weighting 215-318 gms were used for this study. The animals were kept in steel wire cages at the animal house throughout the study, and fed the same type of food to avoid the effect of different food elements on the experiments, the diet consisted of mixed commercial rat laboratory chow. They had free access to water and kept at room temperature. The animals were divided equally into four groups:

G1: This group consisted of 6 male and 6 female rats and served as a control group.
G2: This group consisted of 6 male and 6 female rats in which diabetes was experimental induced by intravenous injection of freshly prepared solution of streptozotocin (STZ) (Sigma, 65 mg/kg, dissolved in 0.2 mmol/l sodium citrate, pH 4.5) (Lutz and Partridge, 1993). For 48 hours after treatment with STZ, the water supply was supplemented with 5% glucose to minimize deaths from acute hypoglycemia (Harry et al., 1993). Within 48 to 72 hours after injection, the criteria of diabetes such as weight loss, glycosuria and hyperglycemia were observed in these rats.

G3 and G4 consisted of 12 male and 12 female diabetic rats for each group in which diabetes was induced as previously mentioned in G2. Diabetic rats were treated with regular insulin (R) (Eli Lilly company, Indianapolis, IN) and NPH (N) insulin (Nordisk Gentofte A/S Laboratorium, Denmark) (2 U R at diagnosis of diabetes and then 1R/3N at 6 PM and 1R/1N at 9 AM daily) subcutaneously for 2 days after induction of diabetes (Sivitz et al., 1998), in G3, and for 21 days in G4.

The initial and final body weights were recorded for rats in all groups (before and after induction of diabetes).

**Sampling Of Blood:**
Blood samples were obtained by decapitation of rats after lightly anesthetized with pentobarbital (90 mg/kg) between 9-11 A.M to avoid the circadian rhythm in serum leptin. Blood glucose was determined in all groups using Reflolux reflectance photometers Boehringer Mannheim UK (Diagnostic &Biochemicals) Ltd. Bell Lanen, Lewes, East Sussex, BN7 1LG GB. The serum was separated by allowing the blood to clot then centrifuged at 3000 rpm for 10 minutes and stored deep frozen at -20°C until used for:


**Statistical analysis:**
All data were expressed as mean ± SE and statistically analyzed according to Yamane (1970). A P value <0.05 was considered significant.

**RESULTS**
Table (1) Fig. (1) illustrate the effect of gender on serum leptin level in
control, diabetic and insulin treated diabetic male and female rats. It was found that the mean value of leptin serum concentration in the control female group (1.80±0.052 ng/ml, P<0.001) was statistically higher than in the control male group (1.25±0.05 ng/ml). However, this difference could not be detected in between male and female in all other studied groups [(0.46±0.042; 0.56±0.066 ng/ml), P>0.05, respectively for diabetic group), (1.02±0.05; 1.1±0.07ng/ml, P>0.05, respectively, for 2 days insulin replacement) and (1.52±0.12; 1.6±0.1 ng/ml, P>0.05 respectively, for 21 days insulin replacement)].

Table (2,3) show serum leptin concentration, insulin level, blood glucose level and body weight in diabetic (untreated) and diabetic treated with insulin replacement for 2 and 21 days in male and female rats compared with the control group. It was found that the mean leptin concentration in both male and female diabetic rats [(0.46±0.042; 0.56±0.066ng/ml, respectively) was significantly lower than in the male and female control groups [(1.25±0.05; 1.80±0.052ng/ml, respect -ively)] (Fig.1). It was observed that the decrease in serum leptin concentration in diabetic male and female rats was significantly corrected with insulin replacement therapy for 2 and 21 days to reach a mean value [(1.02±0.052; 1.1±0.07ng/ml, and (1.52±0.12;1.6±0.1 ng/ml, respectively, P<0.001), nearer to that of normal control rats specially with 21 days insulin replacement. As regards, the changes in serum insulin level, there was no significant difference between male and female control groups, indicating that, insulin may not be responsible for the gender difference in serum leptin concentration observed in Table (1). However, it was noted that the mean value of isulin level in both male and female diabetic groups was lower than that of control male and female groups [(1.5±0.56; 1.36±0.4 μIU/ml respect -ively, for diabetic groups) and [(30.4±1.4; 31.3±2.56μIU/ml, respect -ively, for control groups). Insulin replacement therapy for 2 days and 21 days was found to cause statistically significant increase in serum insulin concen-tration in both male and female rats [(51.4±4.8; 56.4±5.8μIU/ml, respectively, for 2 days group, P<0.001) and (97.4±6.4; 86.3±7.4 μIU/ml, respectively, for 21 days group)] when compared to either control or diabetic groups (Fig. 2). Regarding the changes in blood glucose level, it was found that, there was a statistically significant increase in blood glucose level in diabetic male and female groups[342.7±34.5; 338.5±21.6 mg/dl, respectively], when compared with the control groups [(89.5±4.2; 92.8±4.9 mg/dl, respectively)]. Insulin replacement therapy for 2 and 21 days was associated with a highly significant decrease in blood glucose level in male and female rats [(70.2±5.04, 72.0±4.4 mg/dl, respecti -vely, for 2 days group) and (60.5±2.3, 66.8±2.8 mg/dl, respectively, for 21 days group)], when compared with diabetic groups (Fig. 3). It was also found that, the body weight was significantly decreased in diabetic male and female rats (271.8±6.3, 214.7±7.4gms, respectively). Treatment of diabetic male and female rats with insulin for 2 and 21 days resulted in a significant correction of the reduction in body weight in both male and female rats compared to diabetic groups [(295.7±5.3 ; 235±2.88 gms, respect -ively, for 2 days group) and (297.7±6.02 ; 238.5±2.6 gms,
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respectively, for 21 days insulin replacement group) (Fig. 4).

Leptin concentration, insulin and body weight relationship in the control male and female groups:

A strong positive correlation was observed between leptin concentration and body weight in control male and female rats groups (r= +0.75; + 0.73, respectively, P<0.001).

Moreover, a strong positive correlation was also found between leptin concentration and insulin hormone level in control male (r= +0.76) and in control female (r= +0.9) rats groups (P<0.001). Hence, it is clearly apparent that serum leptin concentration change in a direct proportion to the variation in body weight and insulin hormone serum level in the control group.

Leptin concentration, body weight, insulin and blood glucose concentration relationship in diabetic (untreated) groups:

Serum leptin level showed very highly significant direct correlation with body weight (r= +0.7; + 0.77) and a significant direct correlation with serum insulin level (r= +0.7; +0.71) in male and female groups respectively, and highly significant inverse correlation with blood glucose level in male and female groups (r= - 0.75 ; - 0.71, respectively).

The positive correlation found between leptin concentration and body weight and insulin level in this study (in the diabetic group) revealed a significant rise of leptin concentration with the increase of body weight and serum insulin level. Moreover, leptin was found to be change in an inverse proportion to the variation in blood glucose concentration in both male and female diabetic groups.

Leptin concentration, insulin and blood glucose concentration relationship in the male and female diabetic rats treated with insulin replacement therapy for 2 and 21 days:

Serum leptin level concentration in male groups, showed insignificant direct correlation (r = + 0.41) with serum insulin level and insignificant correlation with blood glucose level (r= - 0.03) for 2 days insulin replacement therapy.

On the other hand, serum leptin level showed very highly significant direct correlation (r= + 0.85) with serum insulin level and highly significant inverse (r= - 0.66) with blood glucose level for 21 days of insulin replacement. While, in female groups, serum leptin level showed highly significant direct correlation (r= + 0.68) with serum insulin level and insignificant inverse correlation with glucose level (r= - 0.26) for 2 days insulin replacement. Moreover, serum leptin level showed very highly significant direct correlation (r= + 0.75) with serum insulin level and highly significant inverse correlation with blood glucose (r= - 0.78) for 21 days insulin replacement.
Table (1): Comparison between serum leptin concentration (ng/ml) in male and female albino rats in all studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Diabetic group</th>
<th>Diabetic treated insulin replacement (2 days)</th>
<th>Diabetic treated insulin replacement (21 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Leptin concentration (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X̄ ± S.E sig.</td>
<td>1.25±0.03</td>
<td>1.80±0.052</td>
<td>0.46±0.042</td>
<td>0.56±0.066</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>1.02±0.05</td>
<td>1.1±0.07</td>
<td></td>
<td>1.52±0.12</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
</tbody>
</table>

P<0.001 = Very highly significant.

n.s. = not significant.

Table (2): Serum leptin concentration (ng/ml), insulin hormone level (μIU/ml), blood glucose level (mg/dl) and body weight (gms) in all studied groups of male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (G₁)</th>
<th>Diabetic (untreated) (G₂)</th>
<th>Insulin replacement 2 days (G₃)</th>
<th>Insulin replacement 21-days (G₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin conc. (ng/ml) X̄ ± S.E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig.</td>
<td>1.25±0.05</td>
<td>0.46±0.042</td>
<td>1.02±0.05</td>
<td>1.52±0.12</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Insulin level (μIU /ml) X̄ ± S.E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig.</td>
<td>30.4±1.4</td>
<td>1.5±0.56</td>
<td>51.4±4.8</td>
<td>97.4±6.4</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Blood glucose (mg/dl) X̄ ± S.E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fig.</td>
<td>89.5±4.2</td>
<td>342.7±34.5</td>
<td>70.2±5.04</td>
<td>60.5±2.3</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Body weight (gms) X̄ ± S.E.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig.</td>
<td>305.3±3.6</td>
<td>271.8±6.3</td>
<td>295.7±5.3</td>
<td>297.7±6.02</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

P<0.01 = highly significant.
P<0.001 = very highly significant.
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**Table (3):** Serum leptin concentration (ng/ml), insulin hormone level (μIU/ml), blood glucose level (mg/dl) and body weight (gms) in all studied groups of female rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control group (G₁)</th>
<th>Diabetic (untreated) (G₂)</th>
<th>Insulin replacement 2 days (G₃)</th>
<th>Insulin replacement 21-days (G₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin conc. (ng/ml)</td>
<td>1.80±0.052</td>
<td>0.56±0.066</td>
<td>1.1±0.07</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>Sig.</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Insulin level (μIU/ml)</td>
<td>31.3±2.56</td>
<td>1.36±0.4</td>
<td>56.4±5.8</td>
<td>86.3±7.4</td>
</tr>
<tr>
<td>Sig.</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>92.8±4.9</td>
<td>338.5±21.6</td>
<td>72.0±4.4</td>
<td>66.8±2.8</td>
</tr>
<tr>
<td>Sig.</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Body weight (gms)</td>
<td>242.7±7.5</td>
<td>214.7±7.4</td>
<td>235.0±2.88</td>
<td>238.5±2.6</td>
</tr>
<tr>
<td>Sig.</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

P<0.01 = highly significant.
P<0.001 = very highly significant.

**Fig. (1):** Serum leptin level (ng/ml) in all studied groups of male and female albino rats.
Fig. (2): Serum insulin level (μIU/ml) in all studied groups of male and female rats.

Fig. (3): Blood glucose level (mg/ml) in all studied groups of male and female rats.
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Discussion

Leptin, a 167 amino acid protein transcribed from the obesity (ob) gene in the adipose tissue, was originally cloned in the mouse during research directed at identifying the molecular defect in obesity-prone strain (ob/ob mouse) (Zhang et al., 1994). The two most important parameters accounting for variations in leptin concentrations are sex and the amount of body fat (Considine et al., 1996).

The present study showed that female rats had significantly higher mean values of serum leptin levels than males in the control group. These data are consistent with results from previous studies in experimental animals (Yacout et al., 200) and humans (Kiess et al., 1998). The gender difference in serum leptin concentrations was also previously described in adults (Danne et al., 1997) and pediatric (Lahlou et al., 1997) healthy subjects, young patients with IDDM (Verotti et al., 1998).

Therefore, it could be concluded that gender seems to be important for the variation in the serum leptin concentration in adult normal rats. However, various factors such as the changes in blood glucose, insulin concentration, and pattern of feeding have emerged which appear to dominate the influence on leptin concentration other than gender. The most likely explanation for this difference is the greater adipose tissue mass in females than males whereas the muscle is greater in males than females. Besides this sex difference in body composition direct influence of sex steroids may be causative (Blum et al., 1997).

Numerous studies have reported the importance of sex steroids to account for the different leptin concentrations according to sex, and the existence of a negative correlation between testosterone -one concentrations and serum leptin concentrations in men (Elbers et al., 1997)

Fig. (4): Body weights (gms) in all studied groups of male and female rats.
and Yacout et al., 2000), although others have not found thin correlation and suggested that, in men, sex hormones are not important independently modifiers of leptin concentrations (Haffner et al., 1997). However, Tuominen et al. (1997) found a negative correlation between leptin and testosterone in a study of male patients with type-1 diabetes mellitus, but not in controls. In the study of Martines et al. (2000), it was found that leptin concentrations in women were more than twice those in men despite the similarity of BMI values in both studied groups. They also failed to find a correlation between leptin concentrations and testosterone in the men with type-1 diabetes mellitus.

It was also found, in the present study, that there is no gender difference in serum insulin concentration in the control group. This finding demonstrates that insulin concentration could not be a factor explaining the gender difference in leptin concentration. In the present study, strong positive correlation was found between leptin concentration and body weight in the control male and female groups. Moreover a strong positive correlation was also found between leptin concentration and insulin hormone level in control male and female groups.

These findings clearly demonstrate that serum leptin concentration change in a direct proportion to the variation in body weight and insulin hormone serum level in the control group i.e. adult normal rats whether male or female, with high body weight or high serum insulin level had a higher serum leptin concentration than rats with low body weight and low serum insulin level.

These findings are consistent with previous studies recorded on the effect of body weight (Considine et al., 1995 and Niklas et al., 1997) and serum insulin level (Havel et al., 1996) on serum leptin concentration.

The present study revealed that streptozotocin induced diabetes resulted in a significant weight loss and a marked reduction in serum leptin concentration which was partially but significantly reversed after 2 days of insulin therapy and completely reversed to baseline (prediabetic) levels or may even overshoot above the base line after 21 days of insulin therapy. These findings are in agreement with the results of Sivitz et al. (1998) who observed that the reversal of the significantly reduced plasma leptin concentrations by insulin treatment of streptozotocin diabetic rats occurred before the return of the significantly reduced body weight or epididymal fat mass to baseline (prediabetic) levels. Hence, they suggested that insulin itself increased leptin concentrations indepently of altered adipose mass.

Havel et al. (1998) found that leptin was markedly decreased in STZ diabetic rats as early as 24 hours to 48 hours after the induction of diabetes, but, before major decrease in body weight. They also, observed that plasma leptin was significantly decreased after 2 weeks and remained low through 12 weeks of STZ diabetes in rats with significantly lower body weight. They reported that the early changes of leptin were proportional to the changes of glycemia but not to changes of body weight. They attributed the weight loss during the first 24 hours to 48 hours after induction of diabetes mostly to loss of body water rather than body fat. In addition, Havel et al. (1998) showed that plasma leptin levels were increased by insulin treatment of STZ diabetic rats in proportion to the reduction of hypoglycemia.

In the present study, low leptin
levels are present in diabetic rats before insulin treatment was begun is also in accordance with that of Kiess et al. (1998) who found that low leptin levels even after adjustment for body mass index (BMI), where present at clinical presentation of diabetes in a small cohort of children and adolescent with IDDM before insulin treatment was started.

There are several possible explanation for this finding: (1) low leptin levels in diabetic animals and/or in-patients with newly onset diabetes might be related directly to the absent or low insulin levels. In fact, data from animal experiments suggest that insulin directly induces leptin expression, pointing to insulin as an important regulator of leptin (Leroy et al., 1996).

In addition, STZ related mice have reduced leptin mRNA levels which are partially restored by insulin treatment (Mizuno et al., 1996). Many investigators reported that the decrease in plasma leptin in STZ diabetic rats was observed to be consistent with the decrease in epididymal adipose tissue leptin mRNA levels (Sivitz et al., 1996 and Havel et al., 1998). Backer et al. (1995) reported a decrease in subcutaneous (inguinal) adipose leptin mRNA in STZ diabetic rats. This decrease in leptin mRNA was not rapidly reversible by insulin therapy and restoration to baseline (non diabetic) levels does not occur in rats prior to restoration of body weight (Baker et al., 1995 and Sivitz et al., 1996). Moreover, Sivitz et al. (1998) observed that the reduced body weight, epididymal fat mass, and epididymal adipose mRNA in STZ-diabetic rats did recover at least, to baseline when insulin therapy was continued for 17 days.

Hence, they suggested that the increase in plasma leptin induced by insulin therapy does not depend on steady state message levels within adipose tissue depots but may depend on posttranscriptional cellular events regulating the translations, intra-cellular pooling, or peptide release or altered clearance of circulating leptin.

Insulin treatment has been previously demonstrated to increase ob mRNA in nondiabetic rats (Saladin et al., 1995) and normalize ob mRNA expression in some (MacDougard et al., 1995) but not all (Kolaczynski and Caro, 1996) studies of STZ diabetic rats. Although, it has been suggested that insulin could have a direct effect on leptin production and/or leptin expression (Caro et al., 1996), studies performed in whole rats and primary rat adipocytes have not always demonstrated a clear insulin effect on ob gene expression in STZ diabetic rats (Kolaczynski and Caro, 1996).

In humans, however, reports on the influence of insulin on leptin are conflicting. While in some studies in human leptin levels were not influenced by hyperinsulinemia (i.e.euglycemia clamp with steady state hyper-insulinemia from 80-120 μIU/ml maintained for up to 5 h) and meal-related increases in circulating insulin levels (Muscelli et al., 1996) although leptin levels showed a definite nocturnal rise in lean, obese, and diabetic subjects. Malmstrom et al. (1996) by using hyperinsulinemic clamps have also shown that insulin significantly stimulated serum leptin concentrations in man (patients with non IDDM).

In the study of Muscelli et al. (1996), the acute administration of insulin in physiological amounts and under euglycemic conditions did not change circulating leptin levels in lean, or obese subjects. Also, the data of Larsson et al. (1996) confirm that it is unlikely that insulin increases plasma leptin even at high circulating levels.
Verroti et al. (1998) showed that leptin concentrations in diabetic patients treated with human insulin were similar to those in non-diabetic subjects. They attributed this finding to adequate metabolic control by intensive insulin treatment. It is well known that in diabetic patients with poor metabolic control, abnormalities of thyroid hormones, growth hormone binding proteins and other hormones are frequently detected (Adcock et al., 1994). In contrast, in diabetic patients with improved metabolic control, normalization of the above abnormalities is generally attained (Caro et al., 1996).

On the other hand, Tuominen et al. (1997) stated that fasting leptin levels were higher in IDDM subjects and remained unchanged during (acute) hyperinsulinemia (i.e. 4h. euglycemic hyperinsulinc clamp). These authors suggested that patients with IDDM are resistant to insulin action on leptin synthesis. The most direct proof, however, that insulin stimulates leptin expression and synthesis in humans stems from in vitro studies with differentiated human adipocytes (Wabitsch et al., 1996).

The present study demonstrated that the increase in plasma leptin in insulin treated STZ diabetic rats was evident as early as 2 days, while the study of Sivitz et al. (1996) showed that leptin mRNA remained well below the prediabetic levels after 2 days of insulin treatment and Backer et al. (1995) found that leptin mRNA in STZ diabetic rats treated with insulin up to 4 days still remained below the levels in non diabetic rats. Hence, in the present study, current 2 days plasma leptin results considered along with past studies of leptin message support the concept of a discordance (disparity between circulating leptin and leptin message (leptin mRNA) in insulin treated diabetic rats).

However, Calpham et al. (1997) measured plasma leptin and subcutaneous adipose tissue leptin mRNA in non-diabetic lean and obese humans before and after a mixed meal and observed no change in either parameter despite a substantial increase in plasma insulin. However, there may be species differences in the rate of leptin response to insulin, or insulin mediated leptin release as, whereas the human studies imply that in vivo leptin responsiveness to insulin requires prolonged insulin administration, the results of the present study together with others (Sivitz et al., 1998) observed a rapid and substantial increase in leptin in STZ-diabetic rats. Certain factors could explain the discrepancy in insulin responsiveness between rodents and humans. It is known that insulin -induced glucose flux, measured as whole body glucose utilization, is higher in rodents than in humans even at equivalent glycemia (Kraegen et al., 1983). Hence, there may be a critical degree of insulin-induced energy uptake beyond which leptin release is triggered.

Dissociation between changes of ob gene expression and changes of circulating leptin concentrations has been reported. For example, plasma leptin increases after increase dexamethazone administration in humans without a corresponding increase of adipose mRNA (Kolaczynski and Caro, 1996).

It should be noted that the circulating insulin concentrations produced by administering exogenous insulin to diabetic rats in these studies were substantially higher than in non diabetic control animals. These levels are, however, necessary to lower plasma glucose concentrations because STZ diabetes is associated with insulin
resistance (Nishimura et al., 1989) in addition to insulin deficiency.

No strict correlation between insulin dose and plasma insulin was detected in diabetic patients because many different factors contribute to the modifications of plasma levels (route of administration, variability of absorption, kinetics of circulation) (Brunetti and Bolli, 1997).

Our study showed a positive correlation between serum leptin levels and serum insulin concentrations in both control and STZ diabetic rats. Several studies have revealed an association between insulin and leptin in both animals (Wabitsch et al., 1996) and humans (Saad et al., 1998), although others found no association between insulin and leptin in obese persons (Dagogo-Jack et al., 1996). In the study carried out by Martinez et al. (2000) in type-1 diabetic adults, no relation was found between leptin concentrations and daily insulin dose. A recent study found that leptin concentrations correlated significantly with insulin dose in prepubertal children, but not during puberty (Moll et al., 1998), although others, did not find this correlation in children with type 1 diabetes mellitus followed from the onset of their diabetes and after several weeks of insulin treatment (Ross et al., 1998). This lack of a significant correlation between insulin dose and leptin concentrations was also noted by Tuominen et al. (1997) in adult patients with type-1 diabetes mellitus.

(2) Alternatively, very high blood glucose concentrations in cases of diabetes mellitus could also suppress leptin levels. However, data regarding a putative regulation of leptin expression by glucose are conflicting. In mice and humans, ob mRNA was inhibited by food restriction associated with glucopenia and was stimulated by injections of glucose in some (Ostlund et al., 1996) but not in other studies (Funahashi et al., 1995).

In the present study, a negative correlation between serum glucose and leptin concentrations was found. Kiess et al. (1998) found no correlation between serum glucose and leptin concentrations in IDDM patients receiving insulin. Verrotti et al. (1998) found no significant correlation between blood glucose level and leptin in either control or diabetics treated with insulin (obese and non obese).

Many physiological studies suggested that variations in blood glucose are not an active signal for leptin secretion (Lahlou et al., 1997). A number of published studies have found that short term insulin adminis-tration does not increase plasma concentrations in human subjects (Dagogo-Jack et al., 1996).

In contrast, some other studies have demonstrated significant increases of circulating leptin concentrations after 4-6 h. of high dose insulin and glucose infusion in non diabetic (Utriainen et al., 1996) and diabetic human subjects (Havel et al., 1996).

Furthermore, infusions of glucose alone, resulting in hyperglycemia, and concomitant endogenous hyperinsulinemia, increases plasma leptin within 4h in humans (Sonnenberg et al., 1996). There is evidence that the induction of marked hyperinsulinemia is not required to increase plasma leptin concentra-tions. For example, low dose glucose infusion sufficient to prevent the decline of plasma insulin and glucose during fasting also prevents the decline of plasma leptin (Boden et al., 1996). In addition, lowering plasma glucose concentrations to euglycemia in hyperglycemic insulin-dependent diabetic human subjects by infusing insulin at
rates that produced physiological insulinemia increases circulating leptin (Havel et al., 1996).

Therefore, an effect of insulin to increase adipose tissue glucose uptake and metabolism, rather than hyperinsulinemia per se may be involved in stimulating leptin secretion (Havel et al., 1998). Consistent with this hypothesis, Mueller et al. (1998) have found that stimulation of leptin secretion by insulin from in vitro cultured rat adipocytes is closely related to the effects of insulin to increase adipocyte glucose uptake. In the same study, blocking glucose uptake with 2-deoxy-D-glucose, phloretin, or cytochalasin-B or inhibiting glucose metabolism with iodoacetate or sodium fluoride produced inhibitions of leptin secretion that were related to decreased glucose utilization, despite the presence of high insulin concentrations. Thus, hypoleptinemia in un-regulated insulin-deficient diabetes may be a consequence of decreased glucose uptake and metabolism in adipose tissue, whereas the restoration of circulating leptin levels by insulin treatment in diabetic animals may be secondary to increased adipocyte glucose uptake and utilization. (3) Diabetic animals before the initiation of insulin treatment had low body weight values that were also significantly lower than body weight values in treated diabetic animals. Thus a reduced body fat mass due to increased lipolysis following insulin deficiency might have resulted in reduced absolute leptin levels in untreated diabetic animals (Kiess et al., 1998). Moreover, assuming that the reduced body weight of diabetic animals was, in part, also due to dehydration (Havel et al., 1998), the calculation of leptin would over-rather than underestimate leptin values with respect to fat mass. Furthermore, the present study, in agreement with that of Kiess et al. (1998), demonstrated a positive correlation between serum leptin levels and body weight or (BMI), and insulin. Sivitz et al. (1998) suggested that the absolute level of adipose cell energy storage may not be as critical for fat cell leptin release as the level of circulating insulin or the rate of insulin-induced energy entry or energy flux. These findings suggest, therefore, that insulin may indeed be an important regulator of leptin levels.

(4) Potential effects of other insulin or glucose responsive factors in modulating the leptin response to insulin in this in vivo study can not be excluded. In particular serum cortisol, which is known to stimulate adipose leptin production, may have played a role (De-Vos et al., 1995). However, this would be more likely to occur in hypoglycemia.

In conclusion, the present study revealed that: 1-Gender is one of the physiological factors which affect circulating leptin level. 2-Since, plasma leptin concentrations are volatile in insulin treated diabetic rats, being markedly reduced under conditions of insulin deficiency and rapidly reversed with insulin treatment, insulin is one of the most important factors in the regulation of circulating leptin level. 3-As leptin concentration was found to correlate directly with insulin level and body weight but inversely with blood glucose level, factors other than insulin are involved in the physiological regulation of circulating leptin concentration such as body weight and blood glucose level.

References

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تأثير السكر المحدث تجريبيا علي مستوى الليبتيه في مصل الدم ودور
العلاج التعويضي
بالأنسولين

نبيل عباس أحمد سليمان
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لقد اكتشف حديثا أن الأنسجة الدهنية تفرز مادة تسمى الليبيتين وقد وجد أنها تقلل
الشهية للطعام وتزيد من استهلاك الطاقة وفي محاولة إلقاء الضوء على بعض العوامل
المؤثرة على إفراز الليبيتين فقد صمم هذا البحث لدراسة تأثير مرض السكر التجريبي
وهرمون الأنسولين على نسبة الليبيتين في حيوانات التجربة من ذكور وإناث الجرذان البالغة
والتي تم تقسيمها إلى أربعة مجموعات تتكون كل مجموعة من 12 جرذًا (6 ذكور و6 إناث).

كما يلي:
المجموعة الأولى: الضابطة
المجموعة الثانية: المصاب بأمراض البول السكري المحدث تجريبيا باستعمال مادة
الستراتونزوتين
المجموعة الثالثة والرابعة: المصاب بأمراض البول السكري المحدث تجريبيا والتي تم
معالجتها بالأنسولين لمدة يومين في المجموعة الثالثة لمدة واحد وعشرون يومًا في
المجموعة الرابعة.

هذا وقد أظهرت النتائج وجود ارتفاع ذو دلاله إحصائية في مستوى تركيز مادة الليبيتين
في مصل إناث الجرذان مقارنة بالذكور في المجموعة الضابطة، بينما وجد أن هناك
انخفاض ذو دلاله إحصائية في مادة الليبيتين في حيوانات التجربة المصاببة بمرض البول
السكري مصحوبة بنقص ذات ذلة إحصائية في تركيز الأنسولين وزن الجسم. وقد لوحظ
عودة تركيز الليبيتين حول معدل الطبيعى مع العلاج بالأنسولين لمدة يومين واحد
وعشرون يومًا.

وقد خلصت النتائج إلى أن هناك عوامل كثيرة تلعب دورها هاما في التأثير على مادة
الليبيتين في مصل الجرذان بالإضافة إلى التغيرات في وزن الجسم والتي يرتبط معها
ارتفاعات طفيفة من هذه العوامل:
1 - النوع (ذكر أم أنثى)
2 - مستوى السكر في الدم حيث وجد أن هناك ارتباطًا عكسيًا بين مستوى السكر في الدم
وتركيز مادة الليبيتين
3 - تركيز الأنسولين في الدم حيث وجد أن هناك ارتباطًا طرديًا بين مستوى الأنسولين
واليبيتين