

**Original article:**

## **Leptin Level in Obese women with and without type 2 Diabetes Mellitus**

**Dr Narayan Chetry, Dr. Syeda Mohsina Rohman**

Dept. Of Biochemistry, GMCH, Guwahati.

Corresponding author : Dr Narayan Chetry

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**Abstract:**

**Introduction:** Leptin is an adipocyte-secreting hormone which regulates appetite and body weight. Leptin, a 16 KDa circulating hormone is a protein made of 167 aminoacids. Leptin functions primarily as an anti-obesity hormone. Its serum concentrations in healthy individuals positively correlate with body fat content, but it correlates negatively when energy intake is reduced and energy stores in fat are declining. The aim of the study is to determine the leptin levels in the obese women in whom type 2 diabetes mellitus were present or absent.

**Methods:** The study was conducted in female patients attending outpatient department, Department of medicine and Endocrinology, Gauhati medical college and hospital, Guwahati, Assam. Forty obese women with type 2 Diabetes (Test group) and forty obese women without type 2 diabetes (Control group) were enrolled in the study. In both the groups, The BMI, WC, HC and WHR were measured. Leptin, HBA1c, Creatinine and lipid profile were measured.

**Observations and Results:** In the present study comparison between the anthropometric, clinical and biochemical characteristics of the control and test group was done. The BMI for the test group and control group was  $31.78 \pm 1.13 \text{ kg/m}^2$  and  $32.51 \pm 1.60 \text{ kg/m}^2$  respectively ( $p=0.022$ ) which is statistically significant. Leptin level in test group ( $20.64 \pm 5.64 \text{ ng/ml}$ ) was lower than in control group ( $29.14 \pm 6.81 \text{ ng/ml}$ ). This difference in mean was found statistically very significant ( $p=0.001$ ). Leptin was well correlated with BMI in test group ( $r=0.476, p=0.001$ ).

**Conclusion:** The mean  $\pm$  SD of serum leptin level in the test group was found to be significantly lower than that of the control group. This difference in serum leptin level between the diabetic obese and non-diabetic obese was explained by altered body fat distribution. Serum leptin levels had a positive correlation with increased total adipose tissue, a known risk factor for type 2 diabetes. Yet the role of leptin in the etiology of diabetes remain unclear. A more elaborate study would have been desirable to precisely establish the role of leptin in diabetes and may help in understanding pathophysiology and perhaps in developing the treatment for diabetes.

Keywords: Leptin, Type 2 Diabetes, Obesity, BMI

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**Introduction**

Obesity is a state of excess adipose tissue mass<sup>1</sup>. Obesity can result from increased energy intake, decreased energy expenditure, or a combination of the two. It is a condition of an abnormal or excessive accumulation of body fat in adipose tissue to the extent that health may be impaired<sup>2</sup>. It is a complex of multifactorial disease that develops from the interaction between genotype and the environment. However, it

involves the integration of social, behavioural, cultural, physiological, metabolic, and genetic factors<sup>3</sup>. Obesity is directly or indirectly associated with various diseases, especially cardiovascular disease, hypertension, diabetes mellitus, sleep apnea, osteoarthritis, fatty liver disease, gallbladder disease, and certain types of cancer. Therefore, its manifestation poses a real threat to health<sup>4</sup>.

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is

underused, producing hyperglycemia. It is classified into two types- type 1 and type 2. In Type 1 Diabetes mellitus there is pancreatic beta cell destruction. This leads to insulin deficiency. In Type 2 Diabetes mellitus (DM) there is decreased ability of insulin to act on the peripheral tissues, this is called insulin resistance. There is beta cell dysfunction which is an inability of pancreas to produce sufficient insulin to compensate for the insulin resistance. Type 2 Diabetes mellitus is the leading cause of morbidity and mortality. Prevention of Diabetes and its associated burden, primarily cardiovascular morbidity and mortality has become a major health issue worldwide.

Leptin, a 16 KDa circulating hormone is a protein produced and released by adipocytes. It is considered to have a role in regulation of body weight and energy metabolism<sup>5</sup>. Leptin is transported into the brain, binds to its receptor in the hypothalamus, and activates JAK-STAT3, leading to suppression of `orexigenic peptides` (e.g. Neuropeptide Y and Agouti-related peptide, which normally increase food intake) and increase in `Anorexigenic peptides` (e.g. Propionmelanocortin and corticotrophin releasing hormones), which normally decrease food intake<sup>6</sup>. At the beginning leptin`s action was thought to be exclusively confined to the CNS. It is now clear that there are multiplicities of peripheral targets organs such as the pancreas, skeletal muscles, liver and gastrointestinal systems<sup>7</sup>.

Leptin together with other molecules that are secreted from the adipose tissue does affect the insulin sensitivity and it is accepted to play a role in the pathogenesis of obesity-related disorders via stimulating vascular inflammation and oxidative stress that may contribute to pathogenesis of atherosclerosis and other cardiovascular complications of obesity<sup>8,9,10</sup>. Approximately 80% of the subjects with type 2 Diabetes mellitus are

obese. It has been found that subjects who have obesity for over 10 years have a two times greater risk for Diabetes than the subjects who have obesity for less than 5 years<sup>11</sup>. After the discovery of leptin secretion from adipose tissue, its role has been more clear in the endocrinology area. Even though leptin limits food ingestion and increases energy expenditure, it has been found high in obesity. This can be explained by the statement that "Obesity may be consequence of leptin resistance(LR)"<sup>12</sup>.

Diabetes Mellitus is a global health problem with increasing incidences in the developing countries. Though insulin is still the mainstay for the treatment of Diabetes because of its life threatening complications, it is high time to look for alternative therapies. In this background the peptide, Leptin could be a potential and beneficial alternative treatment modality that can be thought of with the support of clinical trials for its safety and efficacy. Leptin promotes weight loss, regulation of appetite and can reverse Diabetes by improving glucose intolerance<sup>13</sup>. More importantly, Leptin role in obesity and preventing insulin resistance can go a long way as far as leptin replacement therapy is concerned.

Serum Leptin levels are found higher in woman than men<sup>14,15-18</sup> and this probably owing to adipose tissue in women being higher than in opposite sex. Many investigators have demonstrated that leptin had a major correlation with BMI<sup>13,15-17,19-24</sup>. Some investigators have found that in obese subjects with type 2 Diabetes Mellitus, the leptin levels is characteristically decreased than obese women without type 2 Diabetes<sup>25</sup>. A possible explanation is altered body fat distribution. Subjects with diabetes have increased visceral fat and less subcutaneous fat. Visceral fat produces less leptin than subcutaneous fat<sup>26</sup>. Subjects with Diabetes, therefore, would be expected to have lower

circulating leptin than weight-matched controls as reported in this and other studies.

**Aims and objectives:**

1. To determine the levels of leptin, fasting blood sugar, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, creatinine, glycated hemoglobin in obese women in whom type 2 Diabetes Mellitus is present or absent.
2. To determine the height, weight, waist, hip, waist/hip ratio and BMI in obese patients with type 2 Diabetes Mellitus and controls without having Diabetes Mellitus.
3. To find the possible correlation between these measured parameters and the disease condition under study.

In the current study, We aimed to report the variation of serum Leptin levels in obese women with type 2 Diabetes and obese women without Diabetes and the association between these levels with anthropometric and different Biochemical parameters between the two groups.

**Materials and methods**

The present study was carried out in the Department of Biochemistry of Gauhati Medical College and Hospital, Guwahati. This work has been sanctioned by the Institutional Ethics Committee, Gauhati Medical College, 2013. The Study population comprised of forty (40) obese women with Type 2 Diabetes Mellitus (Case groups) and forty (40) obese women without Type 2 Diabetes Mellitus (Control groups) who sought health care at the endocrinology and medicine outpatient department in Gauhati Medical College and hospital, Guwahat. Selection criteria includes obese women with  $BMI \geq 30 \text{ kg/m}^2$ , Those not receiving Insulin therapy and with intact renal and

liver function and no other major diseases. Type 2DM was diagnosed according to Standards of medical care in Diabetes -2014. This is a case control study wherein a thorough history was taken and detailed physical examination and relevant laboratory investigations were done for all subjects as per the proforma after taking their informed consent. Instructions were given regarding the filing of the Proforma and to come fasting the next morning. Anthropometric examination was done. Five milliliter of fasting blood sample was taken under proper aseptic and antiseptic precaution with a sterile disposable needle and syringe for biochemical analysis.

Estimations of serum fasting glucose, lipid profile (total cholesterol, triglyceride and HDL) and glycated haemoglobin were done using MERCK microlab 300 Semi autoanalyser. VLDL and LDL were calculated using Friedwald's formula. Serum creatinine were estimated using double beam UV Spectrophotometer (Spectra scan UV 2600). Estimation serum leptin were done using ELISA Microplate Reader (Biorad 680). Serum leptin was measured quantitatively by using Origenium's AviBion Leptin ELISA kit. After all the calculations and the biochemical estimations, the results obtained were statistically analyzed and compared between different groups of the study. Baseline characteristics of the study participants are expressed in mean  $\pm$  SD. Leptin levels between the case groups and controls groups were compared via independent t- test. Correlation of leptin with other parameters were evaluated via Pearson correlation analysis. The results were considered significant when the probability (p value) was less than 0.05 % of the observed values of "t" at a particular degree of freedom. Statistical analysis was done using Graph Pad InStat version 3.00. All the statistical graphs were prepared using Microsoft Excel 2007.

**Results and observation:**

Table 1: Shows comparison between the anthropometric, clinical and biochemical characteristics of the control group and the test group.

Parameters	Control Group	Test Group	p-value
Age(in years)	46.13±5.45	47.43±5.69	0.300
Height(m)	1.56±.07	1.544±.05	0.216
Weight(kg)	79.65±9.58	75.97±6.10	0.044
BMI(kg/m <sup>2</sup> )	32.51±1.60	31.78±1.13	0.022
WC(cm)	92.38±4.47	89.58±3.80	0.866
HC(cm)	103.30±3.94	102.10±3.74	0.354
WHR	.892±.029	0.877±.018	0.281
FBG(mg/dl)	89.40±10.23	202.05±90.23	0.116
Glycated hb (%)	5.21±.354	9.12±1.98	0.691
Creat.(mg/dl)	.745±.139	0.699±0.169	0.153
T.chol.(mg/dl)	213.72±34.44	202.70±32.62	0.151
HDL-Cholesterol(mg/dl)	47.08±7.05	33.23±7.10	0.804
LDL-Cholesterol(mg/dl)	130.40±26.87	129.70±24.42	0.772
VLDL-Cholesterol(mg/dl)	36.25±12.43	40.03±16.95	0.747
Triglyceride (mg/dl)	181.35±62.10	200.05±84.50	0.441
Leptin(ng/dl)	29.14±6.81	20.64±5.64	0.0001

Baseline characteristics and biochemical parameters of subjects in the diabetic and control groups are summarized in table 1. The mean age of Test group was 47.43±5.69 years and that of control groups was 46.13±5.45 years (p> 0.05). The BMI for Test group and Control group was 31.78±1.13 kg/m<sup>2</sup> and 32.51±1.60 kg/m<sup>2</sup> respectively (p>0.05). However, the Leptin level in test group was 20.64±5.64 ng/ml and that of control

group was 29.14±6.81 ng/ml (p=0.0001). So, at this statistical level (p=0.0001) of mean values of leptin between the two groups under study was found to be very significant. Comparing height, weight, waist circumference, hip circumference, waist-to-hip ratio, lipid profile between the test group and the control group no significant difference was observed.

**Figure 1: Showing mean values of Leptin in control and test groups.**

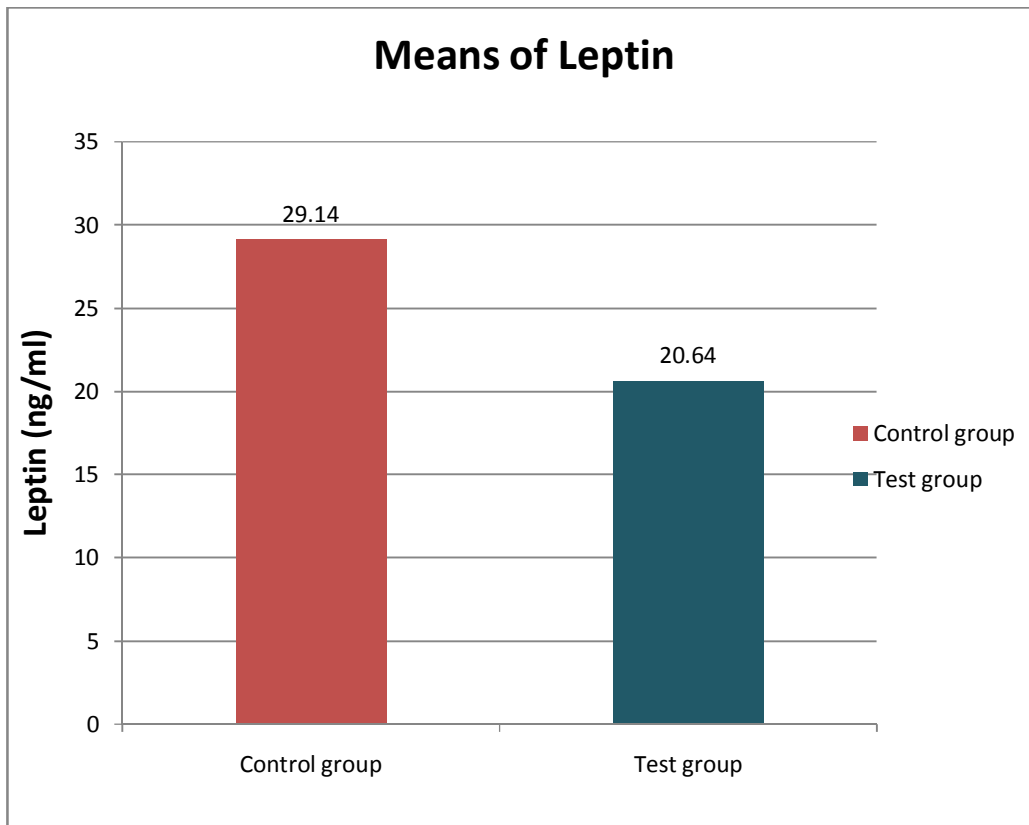


Table 2: shows Correlations (Pearson`s) of Leptin with the various parameters of both the Test group and the Control group.

Leptin Correlations	Test Group		Control Group	
	r	P	R	P
Age(in years)	-0.066	0.683	0.168	0.297
FBG(mg/dl)	-.203	.208	-.202	.212
Glycated Hemoglobin(%)	-.171	.291	-.124	.445
Creat.(mg/dl)	.084	.606	-.044	.784
T.Chol.(mg/dl)	.131	.419	.212	.187
HDL-Cholesterol(mg/dl)	.349*	.027	.093	.567
LDL-Cholesterol(mg/dl)	.006	.969	.171	.292
VLDL-Cholesterol(mg/dl)	.096	.556	.123	.450
Triglyceride(mg/dl)	.095	.557	.124	.443
Weight(kg)	.367*	.019	.354*	.025

Height(m)	.181	.262	.228	.156
BMI	0.476**	0.001	0.415**	.007
WC(cm)	.087	.592	.035	.829
HC(cm)	.018	.910	.072	.661
WHR	.154	.340	.013	.935

\*correlation is significant at the 0.05 level(2-tailed)

\*\*correlation is significant at the 0.01level(2-tailed).

Leptin had a positive correlation with BMI in the test group(  $r=0.476, p=0.001$ ). This correlation between leptin and BMI was found to be statistically highly significant in the test group. The correlation of leptin with BMI in the control group was also found to be statistically significant (  $r= 0.415, p=0.007$ ). Correlation of Leptin was moderate for weight in both the test group and control group. Leptin correlate with HDL-cholesterol in the test group( $p=.027, r=.349$ )

### Discussion

Diabetes Mellitus (DM) and obesity have a complex relationship, with type 2 diabetes strongly associated with obesity. Type 2 DM mainly affects people over the age of 40 and tends to have a gradual course. Leptin, the peptide hormone promotes weight loss, regulation of appetite and can reverse diabetes by improving glucose tolerance. More importantly, leptin's role in obesity and preventing insulin resistance can go a long way as far as leptin replacement therapy is concerned. In this study and other studies like Mehmet Akif Buyukbese et al<sup>27</sup>, it was found that leptin level is lower in obese women with diabetes than without diabetes. Since leptin was lower in obese women with diabetes than without diabetes, we think that further studies are required to make clear the issue for lower leptin levels, whether it is a reason or an outcome. This study is an endeavour towards making even the minutest contribution possible in this upcoming field.

The present study was conducted in the Department of Biochemistry, Gauhati Medical College and Hospital, and it comprised of forty diagnosed type 2 diabetic obese women whose clinical and biochemical findings were compared with forty age matched obese women without type 2 diabetes. The test group which comprised of diagnosed type 2 diabetic patients had mean BMI of  $31.78 \text{ Kg/m}^2$  and mean weight of  $75.97 \text{ kg}$  which were different from the mean of BMI of  $32.51 \text{ kg/m}^2$  and mean weight of  $79.65 \text{ kg}$  in the control group. This difference in mean of BMI and weight between the two groups was found to be statistically significant ( $p=0.022$  and  $r=0.04$ ) respectively. A number of studies confirm gender difference in leptin levels between males and females<sup>28</sup>. Females have higher serum leptin concentrations than males, which confirms the previously reported range in female Kuwaitis<sup>29</sup>. The reason for this is unclear; the larger amounts of body fat mass, predominantly subcutaneous fat, in women and the effect of sex steroid hormones which increase leptin production have been suggested<sup>30</sup>. Since leptin reflects mainly the amount of body fat, this seems to be a logical explanation for the observed gender differences. Gender differences and their relationship to body fat composition were examined in several studies and leptin levels were still found to be significantly higher in women.

In our study, we found that serum leptin levels (mean±SD) in the test group and control group

were  $20.64 \pm 5.64$  ng/ml and  $29.14 \pm 6.81$  ng/ml respectively. The difference between the two groups was highly significant ( $p=0.0001$ ). This finding corroborates with the findings of Farkhanda Ghafoor et al<sup>26</sup>. They reported that obese Pakistani women and men with type 2 diabetes mellitus had lower serum leptin than weight-matched obese controls. It remains to be established whether the lower leptin levels in Pakistani subjects with diabetes are explained by altered fat distribution or relative insulin deficiency or both. A possible explanation of lower leptin levels in diabetic subjects is altered body fat distribution in diabetes. Subjects with diabetes have increased visceral fat and less subcutaneous fat<sup>31</sup>. Visceral fat produces less leptin than subcutaneous fat<sup>32</sup>. Subjects with diabetes, therefore, would be expected to have lower circulating leptin than weight-matched controls as reported in this and other studies. The higher subcutaneous fat content in women would, therefore, explain their higher serum leptin concentrations.

A Minocci et al<sup>33</sup> study found that leptin plasma concentrations were dependent on body fat distribution in obese patients. The result of their study carried out by ultrasound showed that the thickness of abdominal subcutaneous tissue of obese women is greater than in men of comparable BMI, despite higher absolute amounts of total body fat, whereas that of preperitoneal fat is smaller. This study is supported by results of the study from Suzuki et al<sup>34</sup> and by Shimizu et al<sup>35</sup>. Ching-chu Chen, et al<sup>36</sup> study shows that female subjects with type 2 Diabetes mellitus had significantly lower leptin concentration than those in the control group ( $7.8 \pm 5.6$  ng/ml vs  $11.3 \pm 5.4$  ng/ml,  $p < 0.001$ ). Even after adjusting for age, the difference between female subjects with type 2 diabetes mellitus and the control group was still significant in their study. Many studies in the past

have demonstrated that leptin had a major correlation with BMI. In our study, also, leptin had a correlation with BMI both for subjects with diabetes and subjects who did not have diabetes. Leptin had a positive correlation with BMI of the test group ( $r=0.476, p=0.001$ ). This correlation between leptin and BMI was found to be statistically highly significant in the test group. Leptin also had a positive correlation with BMI of the Control group ( $r=0.415, p=0.007$ ). The correlation of leptin with BMI in the control group was also found to be statistically significant. This study corroborates with the study of Mehmet Akif Buyukbese et al<sup>27</sup>. In their study, also, leptin had a significant correlation with BMI both for subjects with diabetes and those without diabetes. In their study mean  $\pm$ SD of leptin in the test group and control group were ( $40.22 \pm 17.77$ ) and ( $50.12 \pm 15.51$ ) respectively and the correlation of leptin with BMI in both the test group and Control group was significant.

Adil Omar Saeed Bahathiq<sup>37</sup> carried out a study on relationship of leptin hormones with body mass index (BMI) and waist circumference in Saudi female population of the Makkah community. He found that leptin concentrations were directly associated with body mass index (BMI) in diabetic obese subjects and showed strong positive correlation ( $r=0.355, p=0.001$ ). In obese subjects without type 2 diabetes, leptin concentrations were directly associated with body mass index and demonstrated a strong positive relation ( $r=0.350, p=0.001$ ). Leptin concentrations were found to be high in both obese and diabetic obese group and showed a positive relation with BMI. It helps in understanding that leptin hormone influences appetite and body weight that cause obesity.

In the present study the body mass index was used as anthropometric measurement for

obesity and fat content. Many studies have confirmed association between BMI and obesity and fat content. We found that serum leptin concentrations were increased in relation to increased body fat content. The positive correlation between body fat and serum leptin is probably explained by the increased release of leptin from large fat cells. Furthermore, leptin can serve as an indicator of fat content and its level may be decreased by reduction of body fat even though BMI values remain unchanged. In fact, some studies have proposed that leptin can serve as an indicator of fat content and that its levels increases exponentially with increasing body fat percentage as mentioned by Concidine et al<sup>38</sup>. A study carried out by Masoud Y Al Maskari, Adel A Alnaqdy found a significant relationship between circulating leptin and BMI<sup>39</sup>, an observation that is associated with the relationship of leptin to total body weight as BMI and fatness. Obesity, itself cannot be defined simply on the basis of method of estimation of BMI alone, therefore we performed waist and hip circumference and the waist-to-hip ratio measurements in diabetic and non-diabetic obese groups. In this study, we found that leptin neither showed any significant correlation with Waist Circumference(WC) ( $r=0.087$ ,  $p=0.592$ ), Hip Circumference(HC) ( $r=0.018$ ,  $p=0.910$ ), waist-hip-ratio(WHR) ( $r=0.154$ ,  $p=0.340$ ) in the test group nor any significant correlation with WC ( $r=0.035$ ,  $p=0.829$ ), HC ( $r=0.072$ ,  $p=0.661$ ) and WHR ( $r=0.013$ ,  $p=0.935$ ).

### Conclusion

The present study was approved by the Institutional Ethics Committee, Gauhati Medical College, Guwahati 2013, vide letter No. MC/233/2013/49. It was a case control study carried out in a population divided into a control group consisting of 40 obese women without type 2 DM and a test group consisting of 40 obese type 2 diabetic women

seeking health care in the department of medicine and endocrinology, Gauhati Medical College & Hospital. Assessment of fasting serum glucose, creatinine, lipid profile and glycated haemoglobin were done in MERCK microlab 300 Semiautoanalyser. Serum leptin were assessed in Biorad 680 ELISA Microplate Reader Version 1.7. Proper consent was taken before the sample collection in all the subjects. Aseptic & antiseptic measures were taken during sample collection to minimize contamination & thereby reducing the error.

The mean  $\pm$  SD of serum leptin level in the test group ( $20.64 \pm 5.64$  ng/ml) was found to be significantly lower than the mean  $\pm$ SD of the control group ( $29.14 \pm 6.81$  ng/ml). The difference between the two groups was found to be statistically highly significant ( $p=0.0001$ ). This difference in serum leptin level between the diabetic obese and non diabetic obese was explained by altered body fat distribution. Leptin, being synthesised by subcutaneous fats, in diabetes it was found that visceral body fats increased relatively compared to subcutaneous fats. The lipid profile: TC, LDL-cholesterol were higher in the control group than the test group, HDL cholesterol was lower in the test group than in the control group. The mean TGL and VLDL cholesterol were higher in the test group than in the control group.

Serum leptin levels had a positive correlation with increased total adipose tissue, a known risk factor for type2 diabetes, yet the role of leptin in the etiology of diabetes remains unclear. Possible explanations are that elevated baseline leptin is a risk factor for future weight and adiposity gain or that elevated leptin suppresses insulin secretion. Insulin estimation is not done in our study. There appears to be a complex, yet incompletely understood, relationship between insulin and leptin.



Though insulin is still the mainstay for treatment of diabetes, because of its life threatening complications, it is high time to look for alternative therapies. In this background, the peptide hormone leptin could be a beneficial alternative treatment modality that can be thought of with the support of clinical trials for its safety and efficacy. Leptin promotes weight loss, regulation of appetite and can reverse diabetes by improving glucose tolerance. More importantly, leptin's role in obesity and preventing insulin resistance can go a long way as far as leptin replacement therapy is concerned. Although great progress has been made in terms of understanding leptin's pathophysiological role, several fundamental questions, particularly regarding the effects of

leptin on glucose metabolism, still remain to be answered.

Above this, Leptin, a adipocyte-secreted hormone that interacts with several organs that are involved in the regulation of energy homeostasis and metabolism. So, leptin treatment in patient with leptin deficiency can correct metabolic abnormalities. Hence, finding from ongoing and upcoming studies combining leptin with other antiobesity medication or sensitizers will be of particular interest. A more elaborate study would have been desirable to precisely establish the role of leptin in diabetes but, due to paucity of time, resource and due to conduction of the study in a sole institution it was not implementable. However, we made a modest effort to fulfill the same through whatever resources available.

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