Effect of plasma-leptin on cardiac disorders with type 2 Diabetes-patients

ABSTRACT

**Background:** The net mechanism by which visceral fat concentration is still unclear because of its interference with autonomic dysfunction which could be simply modified by leptin through the dorosomedical hypothulumus. This work study the potential correlations between visceral fat concentration, cardiac autonomic dysfunction having diabetic disorder (type 2) and leptin.

**Method:** The present work includes 90 cases with cardiovascular risk parameters and diabetic patients and 90 (age- and gender-matched) non diabetic. Typical measurements for cardiovascular risk factors have been measured in addition to plasma visceral fat area, heart variability, leptin and soluble leptin receptor standards.

**Results:** Visceral fat area is highly inversely dependant on the parameters of heart rate variability (p < 0.05and standard deviations of NN (normal RR) intervals during the 24-hour period \( r = -0.239, p =0.003 \). Similarly, the plasma standard of leptin is also important (p < 0.05) showing reverse dependence with the parameters of heart rate variability standard deviations of NN (normal RR) intervals during the 24-hour period \( r = -0.238, p = 0.017 \). In case of non diabetic patients, there are almost zero dependence between leptin and any of heart rate variability parameters.

**Conclusions:** Patients having visceral obesity and type 2-diabetes are strongly affected with hyperleptinemia which may lead to cardiac autonomic dysfunction.

**Keywords:** Visceral fat, Leptin, heart rate, cardiac dysfunction, variability
Leptin is a 167 amino acids (16 kDa-peptide), which controls energy-balance though the hypothalamus[1-4]. However, leptin standards are low in the blood entering brain; while leptin standards are high in the blood leaving the brain leaving. Esler et al [5] have suggested that leptin is prepared in brain tissues and the hypothalamus itself. Moreover, Eikelis et al [6] have reported that in healthy males, about 40% of initial-leptin has an origin from the brain. In addition, the plasma lipoten (PL) levels shared by the brain revealed a 600% increase in over-mass peopele compared with healthy ones. Eikelis et al [6] have reported that the leptin levels in healthy males are 935±32 ng/ml in obese-males against 160±59 ng/ml in healthy-males. The relation between leptin levels and obesity is strong: Several decades ago, obesity has risen globally to be a major reason of serious disorders [7,8]. The accumulation of spare lipid joined by obesity, share in creation of multiple disorders [9]. One can control diabetes, but when sugar standards are out of control it will highly increase the risk of stroke and heart disease. This is why patients with diabetes should keep in mind the permanent risk for developing cvd. Several disorders are created as a direct reason of obesity: 1- High blood pressure which plays a principal risk for cardiovascular disease. Several reports illustrate high relation between insulin resistance and hypertension. If people have on the same time diabetes and hypertension, their risk for cardiovascular disease will increase. 2- Abnormal cholesterol and high triglycerides: Peopele with diabetes have, in general, bad cholesterol standards with high "bad"-cholesterol (LDL); and they have low quantities of "good" cholesterol, and high triglycerides. The famous set of low-lipid counts generally happens with peopele with premature coronary heart disease. This is a character of a dyslipidemia (insulin resistance) accompanied with lipid disorder. 3- Obesity, which is a third principal risk factor for cardiovascular disease and is accompanied with insulin resistance. However, mass-loss decreases the potential risk of cardiovascular disorders, lowers insulin accumulation and rises insulin allergy. High blood pressure in addition with other risk factors will be direct result of insulin resistance and obesity. 4- Physical inactivity is another adjustable principal risk parameter for cardiovascular disease and insulin resistance. Controlling losing weight with permanent exercising can retard, or even prevent, the start of type 2 diabetes, lower blood pressure and help reduce the risk for heart attack and stroke. So, adiposity and cardiac autonomic disorders lead to high probability to get cardiovascular disease in individuals with over-mass. In addition, PL level is, always, correlated to cardiac autonomic dysfunction in patients with type 2 diabetes [10]. In a recent present-work, Siegl et al [11] have reported that hyperleptinemia is a direct consequence of obesity. These authors [11] have also shown that modulates blood pressure
and heart rate are acting directly on the dorsomedial hypothalamus through the sympathetic nervous system, thereby, Ob-R being expressed in obese mice.

Thus far, there are no reports presented that examine the correlations between levels of PL, adiposity, and heart rate variability in cases having diabetes (type-2). This work, reports the correlations that exist between the above mentioned disorders in 90 diabetes-cases was examined, as well as in 90 aged- and gender-matched, non-diabetic individuals having cardiovascular disease risks. Leptin is expressed in not only adipocytes but also mammary epithelial cells and leptin protein is present in milk. Although milk leptin is thought to influence metabolism or the immune system in neonates, there is little information about the regulation of leptin expression in mammary epithelial cells.

PL concentrations are greater in type II diabetic patients and stimulate monocyte chemotactic peptide-1 synthesis via the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway.

**2. EXPERIMENTALS**

The present work concerns studying the clinical factors affect on cardiovascular disorders by clinical observations (atherosclerosis) on 90 non-diabetes Egyptian-patients 90 aged- and gender-matched patients without diabetes as well as 90 Egyptian patients with type 2 diabetes, participated in this work.

All patients who indicated interest to share with the present-work a written reported official-form and the present-work has been approved by the National Research Council of Scientific research at Cairo-Egypt (Contract 0227/ 2015). An initial 90 sequential diabetic patients were included from the cohort, as well as parallel age- and gender-matched non-diabetic patients were consecutively chosen. We have followed the method described in the study of Kadoya et al [12]. The enzymatic method was used to measure serum creatinine. As previously described, an equation for Japanese subjects was used to calculate the Estimated Glomerular Filtration Rate (estimated glomerular filtration rate) for every case.

Heart rate variability has been determined in order to evaluate the cardiac autonomic function, as previously described [13]. According to the testaments for best health usage of heart rate variability (heart rate variability), the two successive R-peak intervals have standard deviation (SD) intervals for each 5-min (Standard deviation of normal R-R intervals recorded for 5 minutes) period have been taken, calculated and estimated. In addition, the
data of Standard deviation of normal R-R intervals recorded for 5 minutes have been used to stand for any variations in heart rate for turning with cycling time more than 5 min, standard deviations of NN (normal RR) intervals during the 24-hour period has been accounted to represent the totality of turning factors which affect the disorder in heart function.

We carried out the clinical data of heart rate variability. The blood-samples were gathered early in the morning taken into account enough fast-period. These samples were sufficiently centrifuged to, finally, have plasma. Using abdominal computed tomography, we obtained a single 10-mm slab from the umbilicus.

3. STATISTICAL METHOD

We estimated the relation between leptin-levels and soluble leptin receptors by using typical-anthropometric techniques. Heart rate variability parameters (standard deviations of NN (normal RR) intervals during the 24-hour period, Standard deviation of normal R-R intervals recorded for 5 minutes), PL and sOb-R levels were logarithm-transformed (log) to achieve normal distribution in the analysis. A non-repeated t test (continuous variables with normal distribution) and Chi square test (for categorical variables) were utilized to compare variables between patients with and without diabetes. To estimate the relationship between heart rate variability parameters and different factors such as PL or soluble leptin receptors, and visceral fat area, we performed simple and multiple regression analyses. heart rate variability parameters were set as a dependent variable in the multiple regression analysis, and after adjusting for age, gender, estimated glomerular filtration rate, hemoglobin A1c levels, duration of diabetes (in affected patients), and presence of hypertension and dyslipidemia, the visceral fat area and PL levels were set as independent variables. All statistical analyses were performed using version 18.0 of the software: statistical package for the social sciences. All reported p values are 2-tailed and were considered statistically significant at a level <0.05.

4. RESULTS

Measurements on diabetic- and nondiabetic patients

Table 1 shows different clinical factors with and without diabetes. Those with diabetes exhibited significantly higher Body Mass Index, estimated glomerular filtration rate, fasting plasma glucose, hemoglobin A1c, visceral fat area, and saturated fatty acid values. While PL, soluble leptin receptors levels, and heart rate variability parameters were not importantly
variant between the various sets, the possible-rate of dyslipidemia and hypertension seem to be feeble in healthy people than diabetic patients.

Table 1. Metabolic factors of diabetic and non-diabetic patients in the present study

<table>
<thead>
<tr>
<th></th>
<th>DM (n = 90)</th>
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<th>NDM (n = 90)</th>
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<tbody>
<tr>
<td></td>
<td>log (SDNN)</td>
<td></td>
<td>log (SDANN5)</td>
<td></td>
</tr>
<tr>
<td>VFA</td>
<td>-0.243</td>
<td>0.015</td>
<td>-0.238</td>
<td>0.017</td>
</tr>
<tr>
<td>SFA</td>
<td>-0.116</td>
<td>0.252</td>
<td>-0.113</td>
<td>0.261</td>
</tr>
<tr>
<td>log (leptin)</td>
<td>-0.243</td>
<td>0.015</td>
<td>-0.231</td>
<td>0.021</td>
</tr>
<tr>
<td>log (sOb-R)</td>
<td>-0.061</td>
<td>0.545</td>
<td>-0.040</td>
<td>0.694</td>
</tr>
</tbody>
</table>

Correlations between visceral fat area, saturated fatty acid, plasma leptin, and soluble leptin receptors levels with heart rate variability factors

For patients with diabetes, visceral fat area, saturated fatty acid, plasma leptin, and soluble leptin receptors levels with heart rate variability factors. In contrast, for non-diabetic patients, these correlations were not found. PL level was inversely related to standard deviations of NN (normal RR) intervals during the 24-hour period and Standard deviation of normal R-R intervals recorded for 5 minutes in diabetic patients. On the contrary, this was not found in healthy group. In either group, plasma soluble leptin receptors level was not significantly correlated to heart rate variability parameters. On the other hand, PL levels were positively related to visceral fat area (in both healthy and diabetic groups). This is shown in table 3 with r = 0.401, p < 0.001, r = 0.259; p = 0.008; and saturated fatty acid (r = 0.729, p < 0.001, r = 0.701; p < 0.001).

This is also shown in figure 1a, plasma soluble leptin receptors level showed the tendency of being related to visceral fat area (r = -0.201, p = 0.070, r = -0.189; p = 0.057), but not with saturated fatty acid, in all healthy and diabetic. Relations between PL levels and heart rate variability factors (0.83 ± 0.41 vs. 0.79 ± 0.42, p = 0.610), standard deviations of NN (normal RR) intervals during the 24-hour period (2.00 ± 0.13 vs. 2.04 ± 0.14, p = 0.165), and Standard deviation of normal R-R intervals recorded for 5 minutes (1.96 ± 0.13 vs. 1.99 ± 0.14, p = 0.183). This did not vary when one compare healthy to diabetic patients: [with (n = 48) and without (n = 52)] neuropathy (in patients with or without diabetic neuropathy PL). In fact, leptin was related to standard deviations of NN (normal RR) intervals during the 24-hour period (r = -0.323, p = 0.02) or Standard deviation of normal R-R intervals recorded for 5 minutes (r = -0.338, p = 0.014), in patients with diabetic neuropathy. On the
other hand, figure 1b shows that leptin is not related to standard deviations of NN (normal RR) intervals during the 24-hour period (\( r = -0.137, \ p = 0.355 \)) or Standard deviation of normal R-R intervals recorded for 5 minutes (\( r = -0.092, \ p = 0.534 \)) for healthy people without diabetic neuropathy.

Independent adiposity and clinical factors with the level of plasma lipten depend strongly on heart rate variability factors

In diabetic patients, multiple regression analyses were performed to study if there are any dependence between PL and cardiac autonomic dysfunction, and what is the extent of the other unexpected-clinical conditions. Several factors affected both standard deviations of NN (normal RR) intervals during the 24-hour period and Standard deviation of normal R-R intervals recorded for 5 minutes: Estimated glomerular filtration rate, presence of hypertension and dyslipidemia, the age, male gender, hemoglobin A1c, and duration of diabetes as covariates, duration of diabetes.

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**Comment [SM11]:** Explain this graph or where do you explain this graph?
The heart rate variability parameters were highly affected with both duration of diabetes and visceral fat area. In model 3, PL stayed highly related to standard deviations of NN (normal RR) intervals during the 24-hour period and Standard deviation of normal R-R intervals recorded for 5 minutes. PL (and not visceral fat area) exhibited important relations with standard deviations of NN (normal RR) intervals during the 24-hour period and Standard deviation of normal R-R intervals recorded for 5 minutes, when both visceral fat area and PL were added to the model 1 covariates (model 4). PL (and neither visceral fat area nor body mass index) stayed strongly related to standard deviations of NN (normal RR) intervals during the 24-hour period and Standard deviation of normal R-R intervals recorded for 5 minutes, when body mass index was included as an additional covariate to model 4. PL stayed strongly related to standard deviations of NN (normal RR) intervals during the 24-hour period (data not shown). This was included as an additional covariate to model 4. Including these compounds, one can consider that the usage of insulin is strongly related to standard deviations of NN (normal RR) intervals during the 24-hour period (β = −0.224, p = 0.034) together with PL (β = −0.256, p = 0.042) and duration of diabetes (β = −0.223, p = 0.041). Thus, PL stayed strongly related to standard deviations of NN (normal RR) intervals during the 24-hour period (β = −0.275, p = 0.033). This was considered when the presence of the cardiovascular disease actions were taken as an additional covariate to model 4 and tended to be correlated to standard deviation of normal R-R intervals recorded for 5 minutes (β = −0.243, p = 0.061). Duration of diabetes and PL revealed a favorable behavior to stay strongly related to standard deviations of NN (normal RR) intervals during the 24-hour period: (β = −0.280, p = 0.062; β = −0.233, p = 0.051, respectively) and Standard deviation of normal R-R intervals recorded for 5 minutes (β = −0.281, p = 0.066; β = −0.207, p = 0.087, respectively). When multiple regression values were re-carried out in 90 non-cardiovascular disease patients (model 4), in contrast, in the non-diabetic patients (data not shown), neither visceral fat area nor PL was independently related to heart rate variability parameters. This is summarized in Table 2:
hemoglobin A1c, presence of hypertension and dyslipidemia, and duration of diabetes as covariates, duration of diabetes was the sole significant factor correlated to standard deviations of NN (normal RR) intervals during the 24-hour period and Standard deviation of normal R-R intervals recorded for 5 minutes.

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 model 2</td>
<td>Both duration of diabetes and visceral fat area were significantly correlated to heart rate variability parameters when visceral fat area was included as an additional covariate.</td>
</tr>
<tr>
<td>3 model 3</td>
<td>PL remained significantly correlated to standard deviations of NN (normal RR) intervals during the 24-hour period and Standard deviation of normal R-R intervals recorded for 5 minutes.</td>
</tr>
<tr>
<td>4 model 4</td>
<td>Both PL and duration of diabetes showed a tendency to associate with standard deviations of NN (normal RR) intervals during the 24-hour period ($\beta = -0.280, p = 0.062; \beta = -0.233, p = 0.051$, respectively) and Standard deviation of normal R-R intervals recorded for 5 minutes ($\beta = -0.281, p = 0.066; \beta = -0.207, p = 0.087$, respectively), when multiple regression analyses were re-performed in 79 non-cardiovascular disease patients.</td>
</tr>
</tbody>
</table>

![Figure 1b: Parameters of heart rate variability: standard deviations of NN (normal RR) intervals during the 24-hour period as a function of PL levels. Note that figure 1a, plasma soluble leptin receptors level showed the tendency of being related to visceral fat area ($r = -0.201, p = 0.070, r = -0.189; p = 0.057$), but not with saturated fatty acid, in all healthy and diabetic. relations between PL levels and heart rate variability factors ($0.83 \pm 0.41$ vs. $0.79 \pm 0.45$).]
0.42, p = 0.610), standard deviations of NN (normal RR) intervals during the 24-hour period (2.00 ± 0.13 vs. 2.04 ± 0.14, p = 0.165), and Standard deviation of normal R-R intervals recorded for 5 minutes (1.96 ± 0.13 vs. 1.99 ± 0.14, p = 0.183).

5. DISCUSSION

Plasma leptin accompanies, always, several parameters: sex, the fasting insulin level and body adiposity which are, in addition, independently related between each other. Moreover, leptin levels are independent on non-insulin-dependent diabetes mellitus; so, impaired leptin secretion does not affect obesity accompanied with non-insulin-dependent diabetes mellitus. Moreover, the pair body adiposity and plasma levels of insulin are affected by insulin sensitivity and not leptin. Several studies have shown that PL levels are strongly correlated with adiposity and its value is correlated to the metabolic-rate of syndrome components [14-20].

One knows quite well that the levels of body mass index, visceral fat area and saturated fatty acid are higher in diabete-patients than healthy. This study shoes that PL concentrations do not vary very much between diabetic-patients and healthy people. However, insulin or C-peptide can alter PL level which may lead to insulin resistance and deficiency.
Table 3. Metabolic parameters correlated with visceral fat area, saturated fatty acid, and sOb-R standards in the present study

<table>
<thead>
<tr>
<th>Variables</th>
<th>log (SDNN)</th>
<th>log (SDANN5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>Age</td>
<td>-0.187</td>
<td>-0.170</td>
</tr>
<tr>
<td>Gender (female = 0, male = 1)</td>
<td>-0.040</td>
<td>0.005</td>
</tr>
<tr>
<td>Hypertension (absence= 0, presence= 1)</td>
<td>-0.103</td>
<td>-0.040</td>
</tr>
<tr>
<td>Dyslipidemia (absence= 0, presence= 1)</td>
<td>-0.136</td>
<td>-0.127</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.140</td>
<td>-0.118</td>
</tr>
<tr>
<td>HbA1c</td>
<td>-0.117</td>
<td>-0.071</td>
</tr>
<tr>
<td>Duration of DM</td>
<td>-0.250†</td>
<td>-0.286†</td>
</tr>
<tr>
<td>VFA</td>
<td>-0.233†</td>
<td>-0.096</td>
</tr>
<tr>
<td>log (leptin)</td>
<td>-0.333†</td>
<td>-0.279†</td>
</tr>
<tr>
<td>Adjusted r²</td>
<td>0.1</td>
<td>0.14</td>
</tr>
<tr>
<td>p value</td>
<td>0.018</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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Comment [SM15]: Superscript and explanation

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Cardiac autonomic system-functions and leptin levels

Few published data have revealed some relation between PL levels and heart rate variability [21, 22]. Moreover, some studies have shown that leptin affects metabolic activities in obese-mice [23-25]. From clinical point of view, PL levels are strongly correlated to adiposity which shows that high values of leptin protein weakened their tendency to restrain feeding and deposition of fat. Lopez [26] has shown that blood pressure and heart rate are directly modulated because of hyperleptinemia. This latter is created directly due to obesity.

In diabetic subjects, only a limited present-work showed significant association of heart rate variability parameters with adiponectin/leptin ratio, in diabetic subjects.

With type 2 diabetic patients, the ratio between leptin and adiponectin, in diabetic-cases, is highly correlated with heart rate variability factors. Moreover, visceral adiposity has a strong relationship with type 2 diabetic patients, including quantitatively determined visceral adiposity. On the contradictory, no net relations were detected between any of the heart rate variability parameters in non-diabetic patients and leptin [27]. Is there are some relations between PL and heart rate variability observed in healthy people? The nature of relation between PL and heart rate variability was only observed in diabetic patients. Potential mechanisms to answer that question: the answer is due to the high values of C6H12O6. Within the activation of the janus kinase 2-phosphoinositide 3-kinase pathway in the pro-opiomelanocortin neuron and the inhibition of gamma-aminobutyric acid (GABA) release to pro-opiomelanocortin neuron in the neuropeptide Y/agouti-related peptide neuron, Leptin activates pro-opiomelanocortin (POMC) neuron. Variation of glucose results in GABAergic synapses to POMC neurons responded differentially to leptin. However, this is not related to hemoglobin A1c level. This work reports that lowering heart rate variability, in diabetic-patients, was related to the duration of diabetes. In addition, perhaps some differences are found due to neuropathy[28, 29]. Several published data [29, 30] revealed that there is not correlation between diabetic neuropathy and leptin. However, somother published works report no correlation between them [16]. These last authors reported that, in diabetic patients, serum leptin level tends to be correlated with sensory conduction velocity. It is more safe to state that leptin is accompanied with heart rate variability only in diabetic patients.

Heart rate variability parameters is affected by adiposity, hypertension, and estimated glomerular filtration rate. Another important parameter that strongly influences the heart rate variability: The body mass index (BMI). High values of BMI in diabetic patients, more than 26 kilograms per meter square results in correlation of leptin with heart rate variability.
Several published works [31-33] have shown that cardiac autonomic function is weakened in patients with cardiovascular disease diseases. However, the similar behavior between leptin and heart rate variability was obtained even after controlling for existing cardiovascular disease disorders.

**Autonomic function and soluble leptin receptors**

The correlation of plasma soluble leptin receptors with heart rate variability was investigated in the present work. Plasma soluble leptin receptors seemed to be inversely related to adiposity; however, it was not highly related to heart rate variability parameters in diabetic patients and healthy people.

**Limitations**

It is important to know that more deep investigations (longitudinal follow-up) for different sets of patients should be carried out to clarify how leptin affect the autonomic dysfunction in patients with and without diabetes.

6. **CONCLUSIONS**

The present experimental data show that:

1- In patients with diabetes, cardiac autonomic dysfunction accompanies always hyperleptinemia, where accumulation of visceral fat is an independent parameter.

2- In particular, in patients with type 2 diabetes, hyperleptinemia is a direct reason for cardiac autonomic dysfunction.

3- Plasma leptin is a reason for pathophysiology of autonomic dysfunction in obesity in humans.

4- Characterizing impaired autonomic nervous system in clinical settings can be carried out with simple electrocardiographic monitoring setup's.

5- Cardiovascular disorders and even mortality can happened due to reduction of heart rate variability, in particular for patients with myocardial infarction and in diabetic patients.

**REFERENCES**


**Comment [SM17]:** This is the important section of the manuscript write in paragraph

**Comment [SM18]:** I will advice you to spend more time on the format the reference according to the specification


Exaplin Model 2  (Log (SDNN) and the variable belows