Evaluation of Blood Levels of Leptin Hormone Before and After the Treatment with Metformin

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Abstract

The purpose of this study was to evaluate the blood level of leptin hormone before and after treatment with metformin. This research is a pre-test and post-test type. The statistical population in this study is 50 patients with type 2 diabetes who referred to Ghods specialized polyclinic in 2017. Patients with type 2 diabetes and BMI> 35 were enrolled. Each patient received 1000 mg of metformin twice daily for 2 months in a pill form, and a blood sample was taken before and after taking the drug and frozen at -20 ° C and, at the time of sampling, to room temperature delivered and measured. Glucose, total cholesterol, triglyceride, cholesterol and cholesterol were measured by common laboratory methods. HbA1c was measured by ion exchange chromatography using the Drew-DS5-UK device. Blood insulin concentration in patients was measured by sandwich ELISA method. Leptin was measured using the sandwich ELISA method. The findings of the study also indicated that body weight, BMI, FFM / Kg, FM / Kg before and after treatment were not significantly different. There was no significant difference between the mean of leptin hormone levels in the pre- and post-test, and there was no significant difference with the concentration of insulin hormone either. But the mean glucose concentration before and after treatment was statistically significant ($p < 0.05$). Also, the results indicated that the effect of drug use in pre-test and post-test on the level of cholesterol, triglyceride and LDL cholesterol levels in the patients was significantly different. In this study, the effect of metformin during the treatment period reduced the blood glucose level of individuals, but its effect on weight loss and HbA1c did not significantly increase due to the duration of treatment.

Key words: Leptin, Metformin, Diabetes.

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Introduction

Obesity is a major public health problem in developed countries. Today, the prevalence of obesity is increasing in developing countries, due to changes in lifestyle, modernization and urbanization. Body mass index (BMI) is a numeric measure that measures the rate of obesity by using weight and height data. BMI does not directly measure body fat, but research has shown that BMI is directly related to body fat (Pevester, 2010). According to the hypothesis of lipostat, that is the leading indicator of the relative stability of body weight, there is a mechanism of post-tracing (negative control) to control eating behavior and increase energy consumption when the body weight gain is of a certain degree (adjustment point); such inhibitory effect will stop when the body weigh falls below this regulatory point (Lindel, 2008). This hypothesis predicts the existence of a post-traumatic message that originates from fat tissue and acts on the brain's control of eating habits and activity. Such an agent was discovered in 1994, and leptin was named. Leptin, a hormone suppressor of eating behaviors when sufficient sources of triacylglycerol were stored, was found to be a factor in the blood of natural mice that, with the elimination of leptin deficiencies, reversed the behavior of the bulky mutated rats (Montsours, 2011). These mutated rats were obese due to overeating, but their body weight was reduced by injection of leptin (Graham, 2012). There are also many causes for obesity, among them physiological causes play a more important role in this regard, with the Kennedy theory of liposuction in 1953 (Brenin, 2008). Based on this theory, fat tissue builds up a substance to regulate body composition, a theory that led to the discovery of a gene that later became known as the "Obesity Gene". This gene is called leptin, and was discovered in 1994 by Zang et al. Leptin comes from a Greek word Leptos, meaning "lean", but it's a bit more complicated than trying to interpret it as leptin is less than fat. Leptin is a hormone that controls appetite and body weight. This hormone is made up of fat cells and sends messages to the brain about how much energy is available to a person. The more fat cells a person has, the more leptin they will have. It seems that when the leptin is not secreted, the brain thinks that there is no fat in the body and it stimulates

the person to eat more and accumulate fat (Enfonds, 2014). The coordination of metabolism in various tissues of a mammal is done by the neuroendocrine system. Adjustment of this system is performed in such a way that separate cells in a tissue sense a change in the current condition and respond by secreting an extracellular chemical message and this message, after attaching to the receptor molecule in another cell, that causes a change in that cell. The hypothalamus of the brain is the center of the neuroendocrine system. Some regulatory mechanisms operate on a very long-term basis and control nutrition and energy consumption in a manner that maintains the body of mammals in a state of equilibrium. A partial imbalance in weight gain can put life at risk. When fat tissue forms a large part of the total body mass, life expectancy decreases. As a result, there is a lot of interest today in researching how to adjust body size and fat content. Leptin was identified as a product of a gene that was shown in laboratory mice with OB (abbreviated to obese, meaning obese). Mice with two defective versions of the gene (ob / ob genotype) indicate the behavior and physiology of animals is in a steady state of hunger: The levels of cortication hormones increase. They are not able to withstand heat, they grow naturally and their appetite is maintained. Due to the latter case, these mice are highly obese and their weight is 3 times higher than normal mice. These mice also have metabolic disorders such as diabetic animals and cannot use insulin (Robbins, 2010). By leptin injection into mutated mice (ob / ob), their weight decreased and locomotor activity increased and their heat production increased. The second gene of the mouse, also labeled DB (for diabetes), has been found in the regulation of appetite. Mice with two defective versions of the gene (db / db) are obese and diabetic. It is known that the DB gene is responsible for coding the receptor for leptin. Leptin activity does not appear when the leptin receptor fails. Leptin is produced only in fat cells and less in the intestinal epithelium and in pairs (Chen, 2013). Leptin receptors are expressed principally in the regions of the brain, including the arcuate nucleus neurons, and the hypothalamic ventromedal, that play a role in regulating eating behavior. This receptor is also expressed in the corpuscular cells of the adrenal glands and the beta panacea cells, albeit at a low level. Leptin carries a message that fat deposits are sufficient and fuel consumption is reduced and energy consumption is rising. Leptin reciprocating with its receptor in the hypothalamus changes the release of an effect on appetite. Leptin also stimulates the sympathetic nervous system and thereby increases blood pressure, heart rate and heat production (producing heat at the expense of metabolic energy) by separating electron transport from ATP synthesis in mitochondria of fat tissue (Yang, 2009). The common model of leptin is a cascade of regulatory events that interact with the interaction of leptin and its receptor and affects the amount of hormones that stimulate or inhibit eating and energy consumption. The amount of leptin released from fat tissue depends on the number and size of fat cells. The leptin that connects to the receptor is as following: The receptor of leptin has a single piece of protein that is circulating in the membrane that is dipped to the outer side by binding leptin. Both monomers of this dimer receptor are phosphorylated by a kinase enzyme. These phosphorous portions act as binding sites for three proteins that are message transducers and transcription activators. Then these three proteins are phosphorylated by the same kinase. Message transducers and transcriptional activators go to the cell nucleus after phosphorylation by kinase and, by binding specific DNA sequences, stimulate the expression of specific target genes. Eventually, the products of these genes affect the nutritional behavior of energy. One of the products of these genes is the α-melanocyte stimulating hormone that acts as an appetite suppressor. The increase in catabolism and the production of heat by leptin is due in part to the increase of the u.p-1 mitochondrial protein in fat cells. Leptin stimulates the synthesis of ucp-1 by altering synaptic transmission of neurons in the arcoat core and hybridization of some hypothalamic neurons. With the creation of a channel, the ucp-1 protein increases the entry of protons into the mitochondrial matrix without passing through the synthase ATP complex. This prevents the oxidation of fuels (fatty acids inside a fat cell) without the synthesis of ATP and releases energy in the form of heat, thus consuming calories or stored fats in large amounts (Freelit, 2010). Metformin is a type 2 diabetes regulator. Metformin has a cell-mediated sensitivity to insulin and anti-hyperglycemia, and is used to treat insulin in Non-Insulin-dependent diabetes mellitus (NIDDM). The precise mechanism of metformin has not been identified, but one of the proposed actions is the clearance of peripheral glucose in low insulin concentrations. Studies indicate that obesity in adults with metformin with type 2 diabetes leads to weight loss, and glucose tolerance and fat stores are corrected. Metformin mainly effects its anti-hyperglycemic effect by reducing glucose output through inhibition of gluconeogenesis. Additionally, the use of metformin in non-diabetic obese adults leads to reduced intake of food and weight loss along with a decrease in glucose, lipids, and insulin in fasting conditions. There are reports that metformin, in addition to the above effects, also reduces plasma leptin levels and fat stores (Jinjirik, 2012).

Methodology

The current study, based on the purpose of the applied type and based on its nature, is a type of pre-test and post-test. The statistical population in this study is type II diabetic patients (including adolescent and youth) referring to Ghods specialized polyclinic in 2012, that is 50 people. In this research, using a simple random sampling method, due to the wide extent of the statistical society and the impossibility of conducting research on the whole society, has been used. Patients with type 2 diabetes and BMI> 35 were enrolled. Each patient received 1000 mg of metformin twice daily for 2 months in a pill from, and a blood sample was taken before and after taking the drug and frozen at -20 ° C and, at the time of sampling, to room temperature, delivered and measured. Glucose, total cholesterol, triglyceride, cholesterol and cholesterol were measured by common laboratory methods. HbA1c was measured using a Drew-DS5-UK device using ion exchange chromatography. Blood insulin concentration

in patients was measured using Sandwich Elise method. Leptin was measured using the sandwich ELISA method. The results of the measured factors were expressed as mean and standard deviation. For statistical analysis, the results of glucose, blood lipids and HbA1c, and leptin and insulin, as well as body weight, BMI, body fat mass, and FFM body mass index from T was used.

Findings and Outcomes

Blood leptin hormone levels were measured in the two experimental groups. The results indicated that the mean of this hormone was 6.5 µg / ml ± 3.3 µg / ml. Also, the amount of insulin was 27.1 ± 28.11 IU / mL. And the mean glucose was 159 mg / dl. Finally, glycosylated hemoglobin was 7.5%. The results indicate an inverse relationship between leptin and insulin showing this difference and the correlation between leptin and insulin, glucose, HbA1c, cholesterol and triglyceride, and BMI in the current study. Table 1 indicates the correlation between leptin and insulin, glucose, HbA1c, cholesterol, triglyceride and BMI.

Also, to evaluate the effect of metformin on weight factors, scores were compared before and after treatment. The results are shown in Table 2.

As shown in Table 2, body weight, BMI, FFM / Kg, FM / Kg before and after treatment were not significantly different. Also, to evaluate the effect of metformin on biochemical and metabolic factors in pre and post test (Table 3).

The results indicated that the mean of leptin hormone levels in pre- and post-test, as well as the concentration of insulin hormone was not significantly different. However, the mean of glucose concentration before and after treatment indicated a statistically significant difference ($p < 0.05$). Also, the effect of drug use in pre-test and post-test on the level of cholesterol, triglyceride and LDL cholesterol in patients was significantly different. Correlation analysis was also used to assess the relationship between leptin and biochemical parameters. The results are shown in Table 4.

The results of Table 4 indicated that there was no significant relationship between leptin of blood and any of the biocompatibility parameters.

Discussion and Conclusion

Obesity is a complex complication characterized by excessive accumulation of fat tissue. Obesity is associated with many health problems, including vascular diseases. The discovery of leptin hormone has led to further research on obesity. The main reason for this progress was that it indicated that the fat tissue of the signals is transmitted to the central nervous system. The Leptin produced by the obesity gene is a protein hormone with a molecular weight of 16 kDa, which is mainly secreted from fat tissue, and has a key role in regulating body weight. It is better to say that leptin acts as a warning mechanism for regulating body fat. This hormone increases energy consumption by increasing the activity of the sympathetic nervous system and lipolysis. Leptin also inhibits appetite by influencing hypothalamic receptors. Therefore, the net effect of leptin is to reduce weight, but deficiency of the hormones or resistance to its effects can both lead to weight gain. Leptin resistance, that is associated with its increase in blood, is much more common in human obesity than the deficiency of this hormone. In recent years, numerous studies have been conducted on the association of leptin with arterial hypertension and heart rate. Leptin independent of CRP, that is an inflammatory marker, is associated with vascular disease, and this finding points to the importance of body fat in cardiovascular problems (Astling, 2011). Leptin receptors are on the endothelium wall and smooth vascular muscle cells. For this reason, leptin imposes intermediate is effective on ability and vascular growth. At the cellular level, leptin stimulates smooth muscle for proliferation. Vascular calcification is accelerated by leptin in empirical models. This hormone increases the oxidative pressure in the vascular wall, which can damage them. Therefore, as a general conclusion, it can be concluded that leptin levels in blood are related to cardiovascular health (Thomas, 2014). The findings also indicated that body weight, BMI, FFM / Kg, FM / Kg before and after treatment were not significantly different. The results of the study indicated that the mean of leptin hormone levels in pre- and post-test patients, as well as in the concentration of hormone insulin no significant difference was found. But the mean glucose concentration before and after treatment was statistically significant ($p < 0.05$). Also, the results indicated that the effect of drug use in pre-test and post-test on the level of cholesterol, triglyceride and LDL cholesterol levels in the patients was significantly different. In this study, the effect of metformin during the treatment period reduced the blood glucose level of individuals, but its effect on weight loss and HbA1c did not significantly increase due to the duration of treatment. Various studies have shown that the effect of metformin has significantly reduced blood lipids, which is consistent with the recent study that indicated that cholesterol and triglyceride and LDL cholesterol decreased after treatment. These findings suggest that the drug has an effect on metabolic pathways in addition to glucose lowering the lipids in these patients. Most studies have shown that a balanced, low-fat diet and physical activity reduce levels of leptin in the blood, even if no significant weight loss occurs. Reduced leptin levels through exercise, changes in energy balance, improved insulin sensitivity and changes in blood lipids are appropriate. The study of changes in leptin with physical activity is one of the issues that is especially important in adolescent and youth obesity. At the same time, there are still no other risk factors for vascular diseases, while many studies point to the onset of dryness and vascular trauma in adolescents.

Table 1: Correlation between research variables

Significance Level	Correlation	Leptin
0/692	0/768	Insulin
0/563	0/326	Fasting Blood Sugar
0/619	0/102	HbA1c
0/594	0/131	Cholesterol
0/804	0/259	Triglyceride
0/561	0/429	BMI

Table 2: Scores of individuals' weight factors in pre and post tests

Significance level	Post- Test		Pre- Test		Weight factors
	SD	M	SD	M	
0/372	17/5	61/6	18/4	64/2	Weight (Kg)
0/242	2/1	29/4	2/5	33/4	BMI
0/827	5/6	44/1	7/3	47/6	FFM/Kg
0/189	4/1	18/9	6/8	21/3	FM/Kg

Table 3: Scores of biochemical parameters in pre and post tests

Significance level	Post- Test		Pre-Test		Biochemical parameters
	SD	M	SD	M	
0/731	1/63	3/2	7/3	1/3	Leptin
0/637	15/5	16/2	4/19	2/15	Insulin
0/002	1/27	173/1	5/61	7/219	Glucose
0/104	1/40	9/9	2/4	4/10	HbA1c
0/009	0/08	214/5	52/4	2/238	Cholesterol (mg/dl)
0/293	0/14	208/4	92/1	1/238	Triglyceride (mg/dl)
0/802	1/06	47/3	25/7	9/51	HDL Cholesterol
0/004	1/93	137/9	62/3	2/163	LDL Cholesterol

Table 4: Correlation between leptin with biochemical parameters

Post-Test		Pre-Test		Biochemical parameters
R	P	R	P	
089/0	867/0	647/0	01/0	Insulin
186/-0	514/0	175/-0	545/0	Glucose
095/-0	762/0	532/-0	02/0	HbA1c
069/-0	731/0	021/0	846/0	Total Cholesterol
217/-0	513/0	034/-0	762/0	Tri Glyceride
199/0	513/0	046/-0	784/0	HDL
/086-0	690/0	-0.051	786/0	LDL
031/0	892/0	198/0	645/0	Body Weight
271/0	539/0	384/0	324/0	BMI
357/0	138/0	512/0	219/0	FFM
305/0	019/0	51/0	029/0	FM

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