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Effects of W100E-Leptin in streptozotocin-induced diabetic mice

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ABSTRACT

Objectives: To determine the effects of W100E-Leptin in a streptozotocin-induced diabetic mice model (effects in the body weight, fasting serum glucose and glucose tolerance).

Methods: Intraperitoneal W100E-Leptin application at 1 mg/kg for 13 days. We used 3 experimental groups (n=6). Group 1: Diabetes + W100E-Leptin (intraperitoneal administration), Group 2: Diabetes + buffer (vehicle) and Group 3: Healthy control + buffer (vehicle).

Results: We determined the effects of W100E on the behavior of the mice, more active, more hair and a tendency to gain body weight. We did not observe any hypoglycemic effect of W100E-Leptin on serum glucose levels in the tests we performed.

Conclusions: These results show us the need to characterize the effects of this hormone in diabetes. We will continue with the characterization of the change that is generated in the protein regulation caused by W100E-Leptin in the diabetes, to propose this hormone as an adjunct against diabetes.

KEYWORDS

Leptin in diabetes, hypoglycemic, adjuvants in diabetes, Leptin exogenous, W100E-Leptin

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by a dysregulation in the metabolism of glucose but also of fat and protein, inducing hyperglycemia, which was caused by impaired insulin secretion, effects of insulin, or both[1,2]. Type 1 diabetes mellitus (T1DM) is a state of total insulin deficiency caused by the destruction of pancreatic cells. Type 2 diabetes mellitus (T2DM) is a chronic systemic metabolic disease characterized by a continuous high level of blood glucose, resistance to the action of insulin, and an inadequate compensatory response to insulin secretion[1,3], some treatments apply insulin to keep blood glucose stable due to progressive failure of pancreatic cells[4]. T2DM and obesity are connected by insulin resistance, which develops unto hyperglycemia through the dysfunction of beta pancreatic cells, caused mainly vía nonesterified fatty acids (NEFAs) that are secreted from the excess of adipose tissue[3].

Although the main characteristic in diabetes is hyperglycemia, alterations in protein metabolism have severe consequences even from the period of insulin resistance, which is reflected in an increase in protein catabolism inducing depletion of skeletal muscle (causing weight loss in diabetic patients) and of other proteins, even when there is an adequate consumption of protein in the diet[1]. While in T1DM this increase in catabolism is at the expense of an increase in protein degradation and not a decrease in protein synthesis, in T2DM it is not easy to determine which processes are involved in the alterations of the metabolism of proteins, although their consequences are less severe[5].

Likewise, due to the increase in the prevalence of obesity, a sedentary lifestyle, unhealthy eating habits and urbanization, the prevalence of type 2 diabetes has also been increasing. The UN estimates that between 2000 and 2030 the total number of people in the world with diabetes will increase by 114%, while the International Diabetes Federation (IDF) predicts that 592 million people will suffer from diabetes in 2035[1,3].

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There are reported papers looking for anti-diabetes drugs, tested in models with streptozotocin-induced diabetes (STZ)[6,7], seeking to determine therapeutic doses with insulin[8], metformin[9], with recombinant Irisin protein[10], and other molecules that seek to promote glucose homeostasis[11].

Leptin Overview

Leptin is a polypeptide hormone that was identified in 1994, composed of 167 amino acids that has a signal peptide of 21 amino acids, which when cleaved gives rise to a mature protein of 146 amino acids with a molecular weight of 16 kDa, mainly produced in adipocytes, the adipokine being mostly secreted by these cells, although it can also be expressed in muscle, mammary gland, placenta, ovaries, skeletal, stomach, pituitary gland and lymphoid tissue[12,13,14,15].

Leptin significantly regulates caloric intake and energy expenditure, and increases glucose metabolism, therefore its deficiency is a cause in the development of diseases and pathological conditions such as obesity, insulin resistance and diabetes, as reported in mice and humans[13,16].

The regulation of energy homeostasis and the control of body weight by means of leptin are carried out at the Central Nervous System (CNS). The leptin receptor capable of activating the signaling pathway after its interaction with leptin, known as ObRb (LepRb), is highly expressed in the hypothalamus, especially in the arcuate, ventromedial, dorsomedial, and ventral premamillary nuclei. Likewise, neurons that respond to leptin are connected to other neurons in the brain, forming an important neural network for the effects that leptin plays. In the arcuate nucleus of the hypothalamus, leptin modulates energy expenditure and food intake through its inhibitory effects on orexigenic hormones such as the Agouti protein-related peptide (AgRP) and the neuropeptide Y (NPY), in addition, leptin it favors the activation of anorexigenic molecules through proopiomelanocortin (POMC) and the α-melanocyte stimulating hormone (αMSH). The participation of leptin in the thyrotropic, gonadotropic, adrenocorticotropic hormone (ACTH) –cortisol and growth hormone axes has also been described, regulating its circadian rhythms, as well as emotional control and memory[13,15].

The effects of leptin at the peripheral level can be brought about by communication of the CNS to peripheral tissues or through direct binding of leptin to its receptors found in these tissues. In skeletal muscle, leptin favors the oxidation of fatty acids and the use of glucose, while in the pancreas, it inhibits the secretion of insulin and glucagon. Likewise, leptin decreases the ectopic accumulation of lipids in the liver and increases the use of glucose by brown adipose tissue; It also increases lipolysis and decreases lipogenesis in white adipose tissue through the sympathetic nervous system. In bone, it increases bone mass and influences other aspects of its metabolism[15].

Due to the effects of leptin on glucose homeostasis, as well as on the regulation of insulin secretion by beta cells of the pancreas, the use of exogenous leptin has been proposed as a possible treatment for diabetes[13,17,18]. However, wild-type leptin (WT) has been shown to have limited solubility at physiological pH, so a replacement for tryptophan at position 100 of the polypeptide chain (W100) by a glutamic acid (E) was performed, to obtain the crystallographic structure of this protein. This leptin variant, which was named W100E-Leptin, has a biological activity similar to leptin WT but with a higher solubility in aqueous solvents[19].

MATERIALS AND METHODS

Expression and purification of recombinant Leptin

Expression and purification of recombinant leptin W100E-Leptin from *E. coli*, recombinant leptin was obtained as has been reported[20]. Briefly, W100E-Leptin was concentrated at 0.5 mg/ml in buffer (50 mM NaH2PO4, 300 mM NaCl, 10 mM Imidazol and pH 8.0) and to use in PBS buffer and pH 7.4.

Preparation of the sodium citrate buffer

Buffer for dilution of streptozocin (STZ). It was obtained by dissolving separately 0.74 g of trisodium citrate dihydrate (Merck KGaA, Darmstadt, Germany) and 0.52 g of citric acid monohydrate (Merck KGaA, Darmstadt, Germany) in 25 mL of deionized water, obtaining two 0.1 M solutions. Subsequently, 15 mL of each solution were mixed for a final volume of 30 mL, adjusting the pH to 4.5[8].

Streptozotocin preparation

STZ (Sigma – Aldrich) was prepared at a concentration of 18.7 mg/mL. We diluted 247.5 mg STZ in 13.2 mL of the sodium citrate solution, the resulting solution was passed through a 0.22 µm filter and stored protected from light and at an average temperature of 4 °C[8].

Animal model

Animal model with high-fat diet consisting of 25% regular chow, 50% lard, 15% sucrose, 5% whole milk powder and 5% yolk, were made in our Laboratory. The experiments were approved and supervised by the local ethics committee in ENMyH-IPN and following the NOM-062-ZOO-1999 (Technical specifications for production, care and use of laboratory animals). 18 mice of the CD-1 strain (8 weeks old, 36 - 41 g), the animals were housed per groups of 3 in cages in a temperature controlled room at 22 ± 2 °C with a 12 h light/dark cycle with free access to food and water[10]. After 1 week of acclimation, the mice were divided into 3 groups, n=6 each one (group 1 and 2 with diabetes and group 3 healthy control with citrate buffer vehicle). In order to induce diabetes, group 1 and 2 were intraperitoneally injected with

streptozotocin (STZ, Sigma – Aldrich) dissolved in citrate buffer (pH 4.5) at a dose of 150 mg/kg[8,10], following 9 days of high-fat diet feeding, while group 3 was fed with same diet, and injected with the equal volume of citrate buffer vehicle. After 9 days post-injection, fasting blood glucose (DAG/FBG) levels were tested, mice with glucose values above 400 mg/dl, were considered diabetic. The group 1/diabetes was treated at doses of 1.0 mg/kg recombinant W100E-Leptin every day by 13 days.

Serum Glucose Level Estimation

Fasting serum glucose levels of control and experimental mice before and after W100E-Leptin treatment were measured by the Accu-Chek Performa (Roche Diagnostics, Mannheim, Germany) digital glucometer (measurement range, 10-600 mg/dL).

Oral Glucose Tolerance Test (OGTT)

After twelve days of treatment, we made an OGTT to resolve the effects of W100E-Leptin on glucose tolerance. To complete this test, we orally administered a single dose of glucose solution (1 g/kg) and W100E (2 mg/kg) to each mouse and measured the subsequent blood glucose levels using an Accu-Chek Performa glucometer at 0 and 2 h after administering the W100E leptin[21].

Statistical Analysis

Results are presented as means and ± SDs. The analysis was executed using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA).

RESULTS

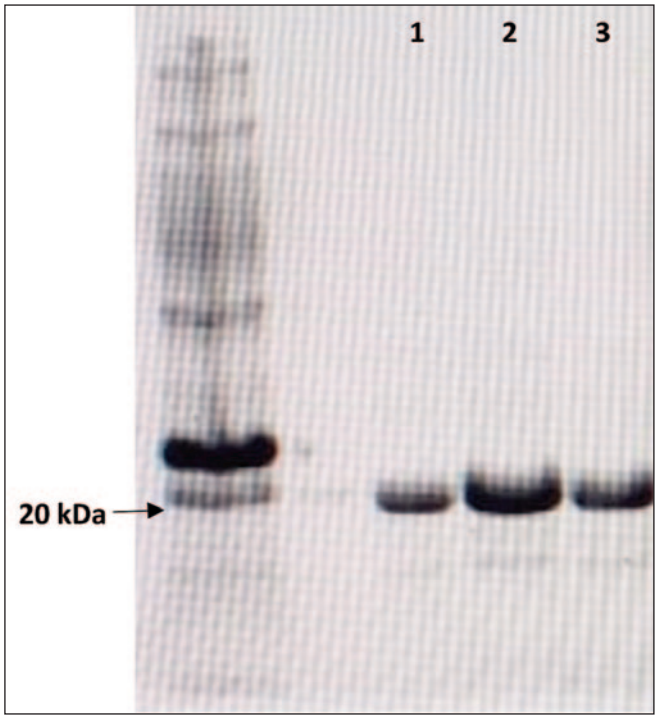
Expression and purification of recombinant W100E-Leptin

SDS-PAGE was used to evaluate the level and purity of W100E-Leptin. There were 3 protein bands with a molecular mass about 21 kDa of W100E-Leptin[20], proteins were stained by Coomassie Blue (Figure 1). The purified protein was kept in 4 °C, PBS buffer, pH 7.4 for the treatment of diabetic mice.

Construction of diabetic mice model

To obtain diabetic mice, CD-1 mice were intraperitoneally injected with 150 mg/kg STZ in a single dose to groups 1 and 2, after 7 days of induced diabetic with STZ, serum glucoses were measured in groups 1 and 2. W100E-Leptin treatment started on the 9th day post-induction, with 12 mice from group 1 and 2 with glucose values of at least 400 mg/dl of serum glucose, measured with the Accu-chek-Performa digital glucometer.

Figure 1. SDS-PAGE of the W100E-Leptin overexpression.



It is shown in lanes 1, 2 and 3, bands of approximately 21 kDa that correspond to the protein with the histidine tract and the cut site.

Bioactivity of W100E-Leptin in diabetic mice

W100E-Leptin effect on body weight

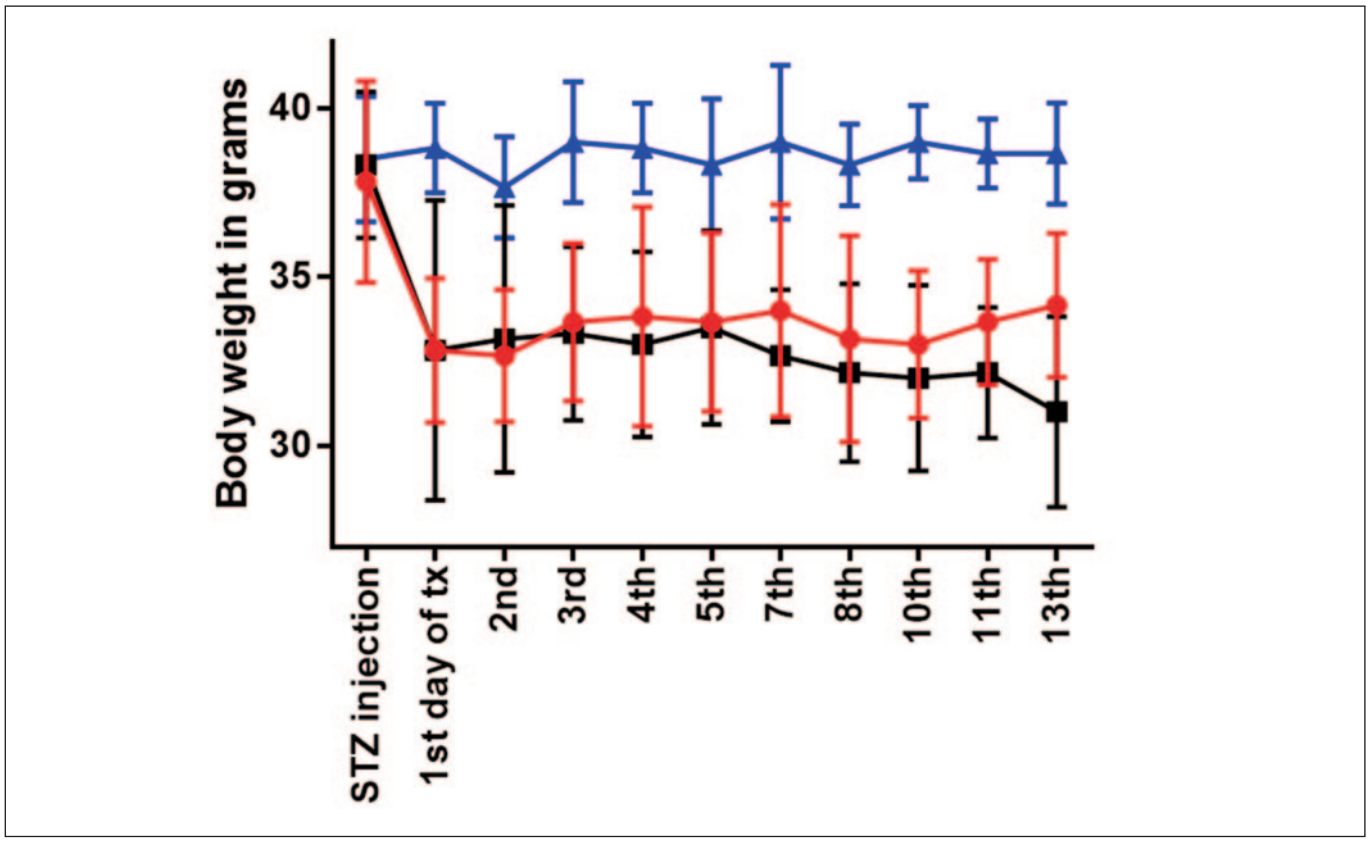
We started treatment with W100E-Leptin on the 9th day of STZ application in groups 1 and 2. We determined that group 1 diabetes with W100E-Leptin for 13 days, maintained their body weight and with a tendency to increase body weight; group 2 with diabetes, application of PBS buffer, behaved with a tendency to lose body weight, as reported[10,22], and healthy group 3, with PBS buffer application, maintained their weight during the 13 days (Figure 2).

Effect of W100E-Leptin on systemic glucose

In the fasting blood glucose (FBG) measurement tests at the beginning and at the end of the treatment, a hypoglycemic effect was not determined, since glucose was measured with an 8-hour fast in the 3 groups of mice. At the beginning and at 13 days of treatment, in which no change in glucose values was found (Figure 3).

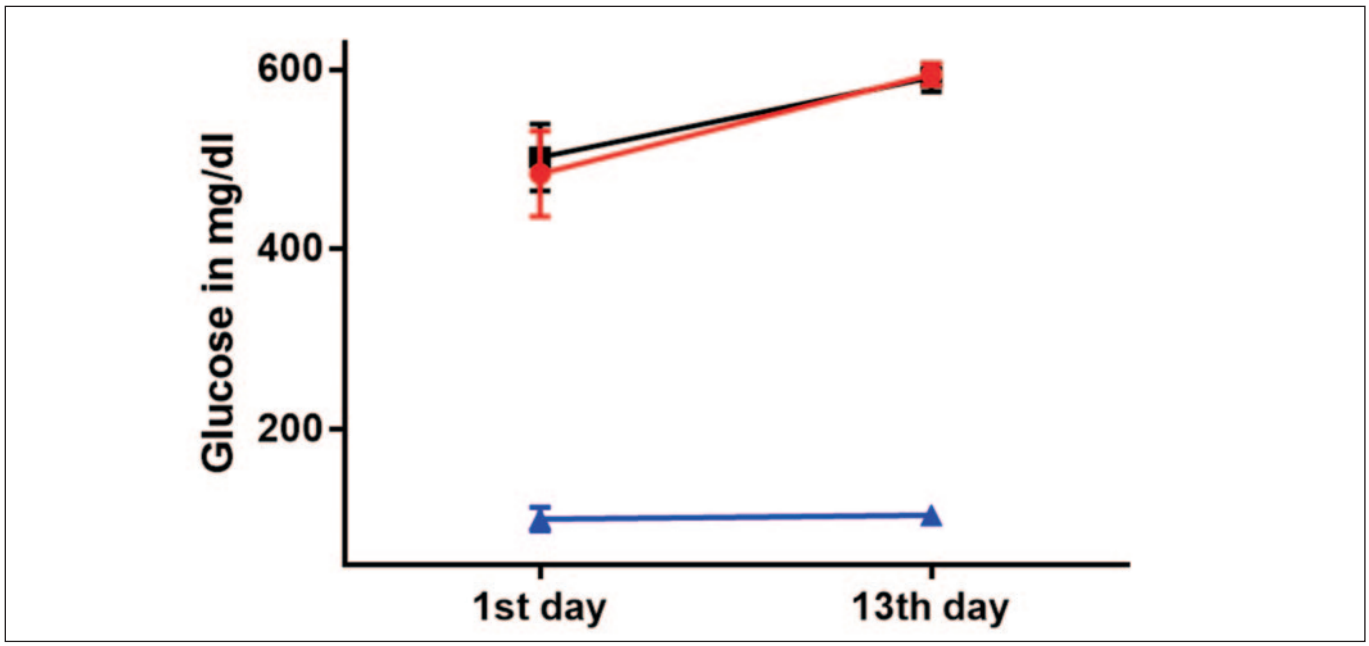
In 2-hour glucose tolerance tests (oral glucose 1 g/kg), we found no hypoglycemic effects caused by W100E-Leptin (ip 2 mg/kg), on the 12th day of treatment the effect was evaluated of W100E-Leptin in glucose values at 2 h after applying the administer glucose orally and W100E-Leptin intraperitoneally. In which no change in glucose values was determined at 2 h (Figure 4).

Figure 2. Average body weight is shown with SD. Weight at the time of STZ application and 9 days after induction of diabetes treatment with W100E-Leptin was started.



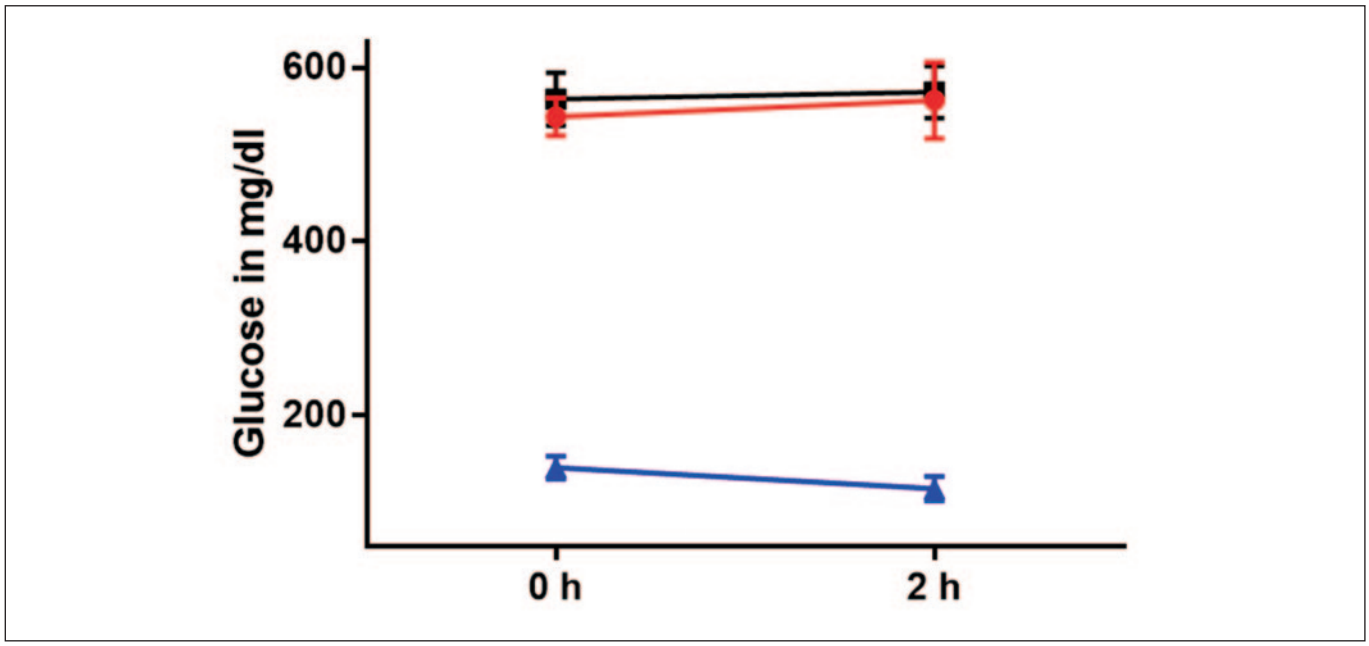
Red: Group 1, Diabetes + W100E-Leptin, Black: Group 2, Diabetes + PBS buffer (vehicle). Blue: Group 3, Healthy control + PBS buffer (vehicle).

Figure 3. Fasting glucose values of 8 h on day 1 and day 13 of treatment.



No statistical difference was determined in the diabetes groups. Red: Group 1, Diabetes + W100E-Leptin, Black: Group 2, Diabetes + PBS buffer (vehicle). Blue: Group 3, Healthy control + PBS buffer (vehicle).

Figure 4. Glucose tolerance test at 2 h. Glucose values when applying leptin and vehicle, and 2 hours after administration.



There is no hypoglycemic effect of W100E-Leptin in 3 groups tested. Red: Group 1, Diabetes + W100E-Leptin. Black: Group 2, Diabetes + PBS buffer (vehicle). Blue: Group 3, Healthy control + PBS buffer (vehicle).

DISCUSSION

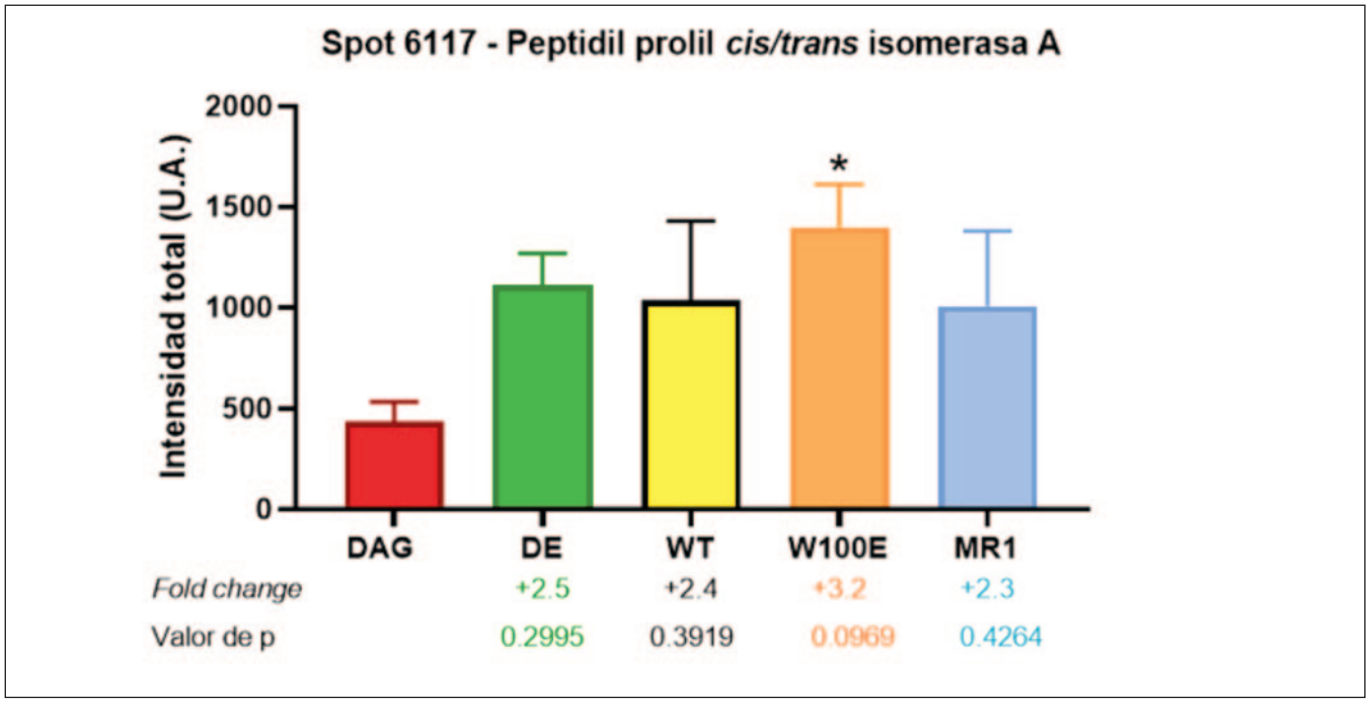
In this study, we focused on determining the effect of W100E-Leptin on glucose values in a mice model with induced diabetes, in which we determined that W100E-Leptin does not have hypoglycemic effects; however, the treatment maintains body weights, since it is reported that in these STZ models of diabetes, the control groups with diabetes lose weight.[10,22]. Furthermore, we identify that the behavior of the W100E-Leptin -treated mice is more active, at the end of the treatment.

Several articles have focused on the effects of exogenous leptin on body weight, demonstrating that this hormone promotes weight loss at the expense of body fat.[23,24,25]; however, our results show the maintenance of body weight in the mice that were treated with W100E-Leptin. The effects of treatment with leptin variants, including W100E-Leptin, on the pancreatic tissue of a murine model that was induced obesity through consumption of a high fat diet (HFD) for 4 weeks were analyzed in one study. In the group that was fed with HFD and that did not have treatment, there was an increase in glucose serum concentrations, while those who were fed with HFD and received treatment with leptin variants observed a change in the values of triglycerides, cholesterol and other proteins[20,26]. Likewise, 15 proteins that were regulated by these leptin variants were identified, among which were found peptidyl prolyl cis / trans isomerase A (Figure 5), elongation factor 1-delta (Figure 6) and ribosomal protein 60S- P0 (Figure 7), which are involved in protein synthesis and folding. In the untreated high fat diet (HFD) group, it was observed that these proteins are overexpressed compared to the standard diet (DE) group, which was the healthy group, and that after treatment with the leptin variants, but especially with the W100E-Leptin variant, their levels were restored[27]. These results indicate that due to the stress resulting from obesity and hyperglycemia, there could be a decrease in cellular metabolism, which includes a decrease in protein synthesis giving priority to the synthesis of acute positive phase proteins and other proteins, they have an important role in inflammation processes. Likewise, in this study it was proposed that these alterations in protein synthesis could result in an increase in apoptosis of pancreatic cells, aggravating the picture of diabetes[27].

The results of this study suggest that exogenous W100E-Leptin could help maintain an adequate rate of protein synthesis, which prevents protein loss due to the increased catabolism of muscle proteins and, therefore, the conservation of lean mass, in fact, other studies showing that leptin stimulates skeletal muscle growth[28]. This may explain why mice treated with exogenous W100E-Leptin maintain their body weight in this study compared to those who did not receive the treatment[29].

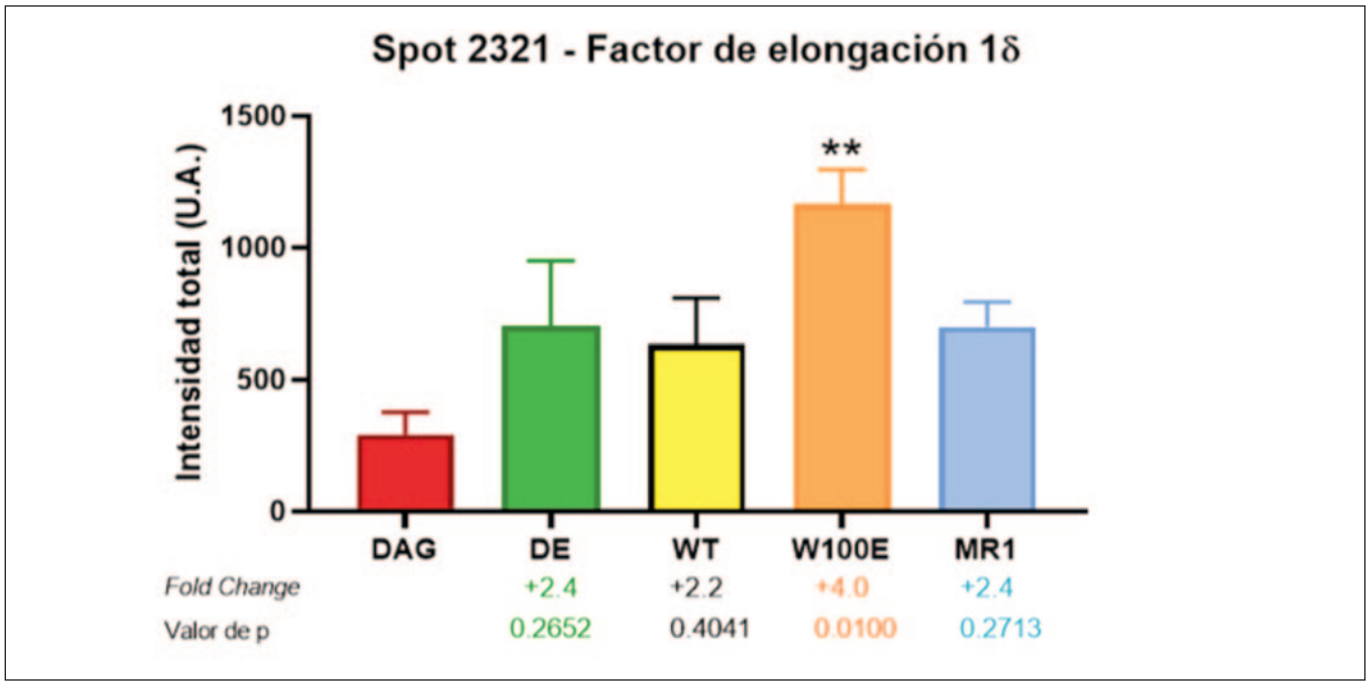
On the other hand, several research groups have devoted themselves to analyzing the effects of leptin on mood, it is reported in a study in humans, where was analyzed the relationship between endogenous leptin concentrations according to their circadian secretion and emotional state, finding that

Figure 5. Changes in the expression level of peptidyl prolyl cis/trans isomerase A (PPIA) due to the effect of high fat diet (DAG/HFD) and treatment with leptin variants at a dose of 1 µg/g/d for 4 weeks.



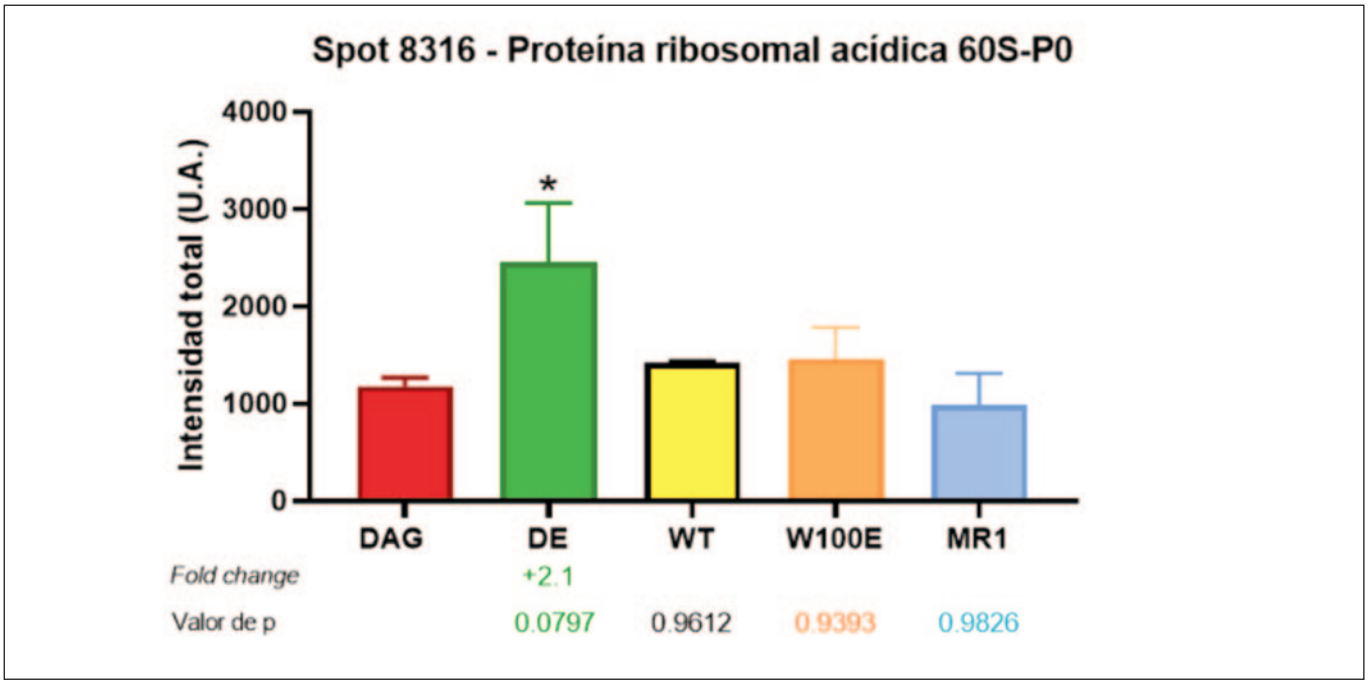
Data are expressed as the mean ± SEM (n = 3). 1-way ANOVA, followed by a Dunnett comparison, * P <0.1 vs DAG. The fold change and P values are shown below the experimental groups according to the results obtained by the PDQuest ™ software. (Bio-Rad) and by statistical analysis, respectively[27].

Figure 6. Changes in the expression level of the 60S-P0 acidic ribosomal protein due to the effect of high fat diet (DAG/HFD) and treatment with leptin variants at a dose of 1 µg / g / d for 4 weeks.



Data are expressed as the mean ± SEM (n = 3). 1-way ANOVA, followed by a Dunnett comparison, * P <0.1, ** P <0.5 vs DAG. The fold change and P values are shown below the experimental groups according to the results obtained by the PDQuest ™ software (Bio-Rad) and by the statistical analysis, respectively[27].

Figure 7. Changes in the expression level of the 60S-P0 acidic ribosomal protein due to the effect of high fat diet (DAG/HFD) and treatment with leptin variants at a dose of 1 µg / g / d for 4 weeks.



Data are expressed as the mean ± SEM (n = 3). 1-way ANOVA, followed by a Dunnett comparison, * P <0.1 vs DAG. The fold change and P values are shown below the experimental groups according to the results obtained by the PDQuest ™ software (Bio-Rad) and by the statistical analysis, respectively[27].

during the periods in which they had higher levels of circulating leptin, the study subjects felt happier and presented a less tendency to social interaction, while in periods of lower circulating leptin concentration feelings of sadness and social interaction predominated[30], also, was observed that in rats with unpredictable chronic stress there is a reduction in leptin levels, and that when administering exogenous leptin the symptoms of depression decreased. Likewise, they observed that after the administration of exogenous leptin there was an increase in the levels of c-fos mRNA, a marker of neuronal activation, in the CA1 and CA3 regions of the hippocampus as well as in the dentate gyrus[31]. Depression has also been reported to be characterized by disturbances in neurotransmitters such as dopamine, 5-hydroxytrypatin (5-HT), and gamma-aminobutyric acid (GABA), and that leptin increases expression levels and transport of 5-HT, and improves mood through the dopamine pathway[32].

CONCLUSION

In this study the effect of the recombinant W100E-Leptin was determined in a murine model with induced diabetes, we determined an effect on the behavior of the mice, more active with more hair and a tendency to gain body weight. We did not observe any hypoglycemic effect of W100E on serum glucose levels in the tests we performed. These results show us the need to characterize the effect of this hormone in diabetes, so we will continue with the characterization of the change that is generated in the protein regulation caused by W100E-Leptin in the diabetes model by STZ and propose new effects caused by this hormone, propose the impact on diabetes and be able to propose this hormone as an adjunct against diabetes.in which we did not find any hypoglycemic effect of W100E-Leptin on the serum glucose values, since in the groups with diabetes and healthy control.

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