Research Article

Effect of Opioid Receptors Modulation on the HFD-Induced obesity in Adult Male Albino Rats

Ibrahim Y. Ibrahim, Hanaa M. Ibrahim, Neven M. Aziz, and Doaa M. Abdel Rahman
Department of Physiology, Faculty of Medicine, Minia University, Minia, Egypt

Abstract
Aim of work: The involvement of the opioid system in energy balance has been known for several decades but many questions remain unanswered. Therefore, this study was designed to investigate the effect of the opioid receptor agonist (Tramadol) and antagonist (LY٢٥٥٥٨٢) on high fat diet (HFD) induced obesity for ٤ weeks. **Materials and Methods:** Thirty two adult male albino rats were divided into ٤ equal groups: Control, HFD, HFD+LY٢٥٥٥٨٢ treated and HFD+Tramadol treated groups. LY٢٥٥٥٨٢ (٠.٣١ mg\kg, sc) and tramadol (٠.٥mg\kg, orally) were administrated daily with HFD feeding for ٤ weeks. Blood samples were collected for measurement of lipid profile, glucose, insulin and leptin. Body weight, body mass index (BMI), and food intake were also measured. **Results:** Consumption of HFD resulted in a significant increase in body weight, BMI, serum glucose, insulin, leptin levels and induced a state of dyslipideamia. Opioid antagonist; LY٢٥٥٥٨٢ administration with HFD decreased food intake, body weight and BMI, in addition to the improvement of HFD related metabolic abnormalities (dyslipidemia and insulin resistance). On the other hand, treatment with the opioid agonist; tramadol caused marked decrease in food intake, body weight, and BMI, but with no significant effect on the HFD induced metabolic disorders. **In conclusion,** opioid antagonists could be considered a promising approach in management of HFD-induced obesity.
Key Words: obesity, high fat diet, BMI, leptin, insulin, glucose and opioid receptors.

Introduction
Obesity is a pathological condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems [١].

The endogenous opioid system consists of the endogenous opioid peptides and their corresponding receptors by which these peptides produce their effects. The primary central nervous system (CNS) opioids (β-endorphin, enkephalins, and dynorphins) are agonists for mu (μ), kappa (κ), and delta (δ)-opioid receptors. Stimulation of the central μ-opioid receptor by infusion of opioid peptides including β-endorphin or synthetic agonists has been found to increase body mass and food intake[٢].

Opioid receptors exist not only in the nervous system, but also in peripheral organs, such as heart, lungs, liver, gastrointestinal and reproductive tracts[٣]. In the GI tract, opioid receptors are present in the smooth muscle cells and at the terminals of sympathetic and sensory peripheral neurons[٤].

Control of food intake is regulated by a complex system of central and peripheral signals which interact to modulate the individual response to food intake. Peripheral regulation includes satiety signals via hormones secreted from GIT and adiposity signals, while central control includes many factors which are neuro-peptidergic, monoaminergic and endocanna-binoid system[٥].

The brain opioid system has a role in regulating food intake and in mediating the rewarding impact of palatable food intake and the modulation of opioid receptors can

interfere with the control of food intake. Within the hypothalamus, both μ and κ receptors stimulated agouti-related peptide (AgRP) and neuropeptide Y (NPY) induced food intake. Orexin-induced feeding behavior was also stimulated by opioids. In addition, endogenous opioids regulate the mesolimbic dopamine pathway, and activation of opioid receptors in this area stimulates food consumption, whereas their blockade suppresses feeding behavior [٦].

On the other hand, the peripheral endogenous opioids mediate suppression of gastro-intestinal neural activity, inhibition of gastric emptying, delay of gastro-intestinal transit and satiation *via* peripheral μ-opioid receptors[٧]. Thus, the endogenous opioids, both centrally and peripherally, could affect food intake, which suggests an important role for endogenous opioid peptides in the control of body weight. Now the question is "Which of these mechanisms of opioid action predominates, the central mechanism mediating inhibition of satiety or the peripheral mechanism that mediate satiation?"

The role of opioids in the regulation of food intake is controversial: in general opioid agonists enhance feeding and opioid antagonists decrease feeding but some studies showed that opioid agonist can decrease feeding depending on the dose and dosing regimen[٨]. Therefore our work aimed to study the effect of an opioid receptor agonist; Tramadol and an antagonist; LY٢٥٥٥٨٢ on HFD induced obesity in adult male albino rats with its metabolic abnormalities during the dynamic phase of obesity development.

Materials and methods

I- Animals

Thirty two adult male albino rats (Sprague dawley strain) were used. Their weight ranged between ١٥٠-١٨٠ grams at the beginning of this study. Rats were housed in stainless steel mesh cages offering individual housing. They were housed at room temperature with natural light\dark cycles for one week for acclimatization to lab conditions. Rats were fed a standard diet of commercial rat chow and tap water *ad libitum* until the time of the experiment. All the procedures applied to rats were in accordance with our institutional guidelines. The protocol was ethically approved by The Laboratory Animals Maintenance and Usage Committee of Faculty of Medicine in Minia University.

During the acclimatization period, daily food intake was measured to know the mean daily food intake per rat. Rats were randomly divided into ٤ main groups (٨ rats each):

١- ***Control group (C):*** in which rats were fed a commercially available standard diet for ٤ weeks.

٢- ***HFD group:*** in which rats were fed high fat diet (HFD) for ٤ weeks.

٣- ***HFD + LY٢٥٥٥٨٢ treated group (HFD+LY group):*** in which rats were fed a HFD with concurrent daily subcutaneous injection of LY٢٥٥٥٨٢ in a dose of ٠.٣١ mg\kg body weight for ٤ weeks [٩].

٤- ***HFD + Tramadol treated group (HFD+T group):*** in which rats were fed a HFD with concurrent receiving tramadol by oral tube once daily in a dose of ٠.٥ mg\kg for ٤ weeks[١٠].

At the end, the rats of all groups were fasted for overnight and then decapitated. After collection of blood from jugular vein, the samples were left to clot at room temperature, and then centrifuged at ٣٠٠٠ rpm for ١٥ min in a centrifuge (Hettich centrifuge). The serum layer was then withdrawn into identified eppendorf tubes and stored at - ٢٠ °C till the time of assay.

II- Drug protocol

LY٢٥٥٥٨٢ powder ***(Sigma- USA)*** is a centrally active non selective opioid receptor antagonist [١١] and it was dissolved in water acidified with ١٪ lactic acid.

Tramadol ***(October pharm-Egypt)*** is a non-selective opioid receptor agonist [١٢] and it was dissolved in water.

III- Diet protocol

The composition of standard diet (g/kg diet) was according to the formula of Davidson et al.,[١٣] that contained (Fat ٥٪ [corn oil

٥٪], carbohydrates ٦٥٪ [corn starch ١٥٪ and sucrose ٥٠٪], proteins ٢٠.٣٪ [casein ٢٠٪ and DL-Methionine ٣٪], fiber ٥٪, salt mixture ٣.٧٪, and vitamin mixture ١٪) and provided ٣.٠ kcal/g of diet. On the other hand, the HFD contained ٢٠ g of fat/١٠٠ g of diet (١٩ g of butter oil and ١ g of soybean oil to provide essential fatty acids) and provided ٤.١ kcal/g of diet[١٤].

Standard diet was purchased from El-Gomhoria Company, Cairo, Egypt, while high fat, diet was prepared manually and stored at ٤°C until used. The daily food intake was measured for each group. Individual body weight of rats in each group was assessed once a week.

IV- Body mass index (BMI)

Body length (nose-to-anus length in centimeter) was determined in anaesthetized rats with light ether. Rats were also weighed weekly in gram using electronic balance (FY ٢٠٠٠). The body weight and length were used to determine BMI according to the following formula:-

Body mass index (BMI) = body weight (g)/ length^{٢} (cm^{٢}).

BMI is used for comparison between rats as considered by Novelli et al.,[١٥] a significant increase in BMI in comparison to a control is a marker of obesity and also obesity is usually taken as any significant increase in body weight or energy content relative to control animals**.** This was also according to Li et al.,[١٦].

V- Biochemical analysis

Total cholesterol (TC), triglycerides (TGs), low density lipoprotein (LDL-c), high density lipoprotein (HDL-c) and glucose were determined by enzymatic colorimetric methods, using kits purchased from Bio-diagnostic, EGYPT. Serum insulin and leptin were determined by enzyme-linked immunosorbent assay (Glory).

Statistical Analysis

Statistical analysis was performed using Graph pad Prism ٥ software and significant difference between groups was done by one-way ANOVA followed by Tukey-Kramar post hoc test for multiple comparisons with a value of P ≤٠.٠٥ considered statistically significant.

Results

In the present study, there was no significant difference between the control, HFD, HFD+LY and HFD+T groups as regard body weight, BMI and food intake at the start of experiment.

The results of the present study revealed that the HFD group showed a significant higher food intake in the first and second weeks as compared to the control group. There was also a significant lower amount in the final food intake in HFD group as compared with the control group and with its initial week (Table ١). This was accompanied with significantly higher values of the final body weight (Figure ١) and BMI (Figure ٢) as compared to the control group and with its initial values. In addition, there were significantly higher levels in the total cholesterol, TGs, LDL-c, serum glucose, insulin and leptin with a significant lower level of HDL-c as compared with the control group (Table ٢).

Injection of LY lowered significantly the body weight from the second week up to the end of the study as compared with control and HFD groups. In addition, tramadol treatment also significantly lowered the body weight as compared to the control, HFD and HFD+LY groups from the start up to the end of the study. The final body weight in the treated groups was significantly decreased as compared with its initial value (Figure ١). As regard final BMI, there was a significant lower value in the treated groups than that of the control, HFD groups and its corresponding initial value. Treatment with tramadol also significantly lowered the final BMI when compared to the HFD+LY group (Figure ٢).

In the present study, treatment with LY significantly lowered the amount of the final food intake as compared with control, HFD groups and with its initial week, while tramadol treated group showed a significant increase in the food intake in the first week as compared to the control, HFD and HFD+LY groups then showed a marked

significant drop in the second, third and fourth weeks as compared to all studied groups and with its initial week (Table ١).
There were significant lower values in the total cholesterol, TGs, LDL-c with a significant higher level in HDL-c in the HFD+LY group as compared with control and HFD groups. Additionally, LY treatment significantly lowered serum levels of glucose, insulin and leptin as compared to the HFD group but insignificantly different from the control group. On the other hand, treatment with tramadol failed to produce any significant change in the serum levels of TGs, HDL-c, LDL-c, glucose and insulin but with a significant higher TC level as compared with HFD group and this was associated with a significant lower leptin level when compared with all studied groups (Table ٢).

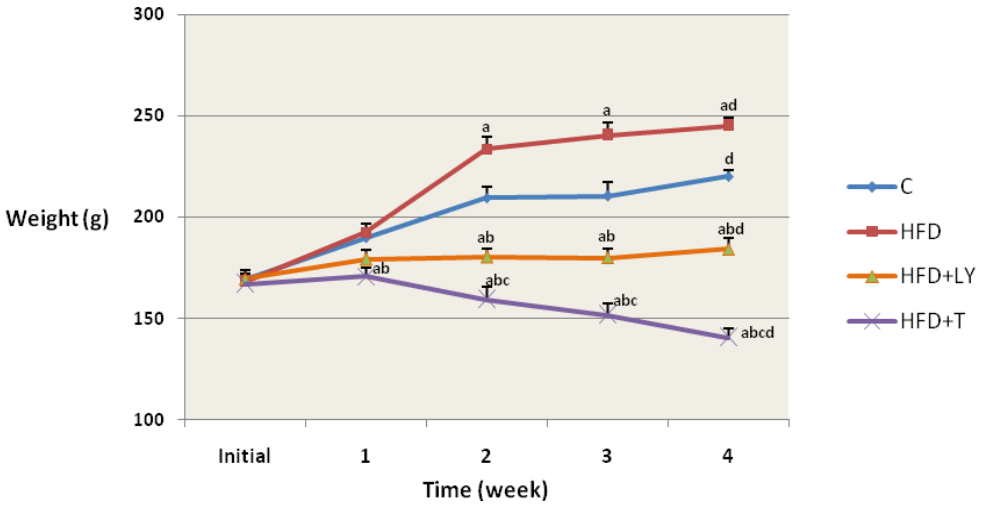

Figure (١): Time course changes in the body weight (g) in different studied groups. HFD: high fat diet, HFD+T: HFD+Tramadol treated group. a: Significant from control group (C), b: Significant from HFD group, c: Significant from HFD+LY (HFD+LY٢٥٥٥٨٢ treated group), d: Final value significant from its corresponding initial value, $P< ٠.٠٥$. Data are expressed as mean ± S.E.M. of ٨ rats in each group.

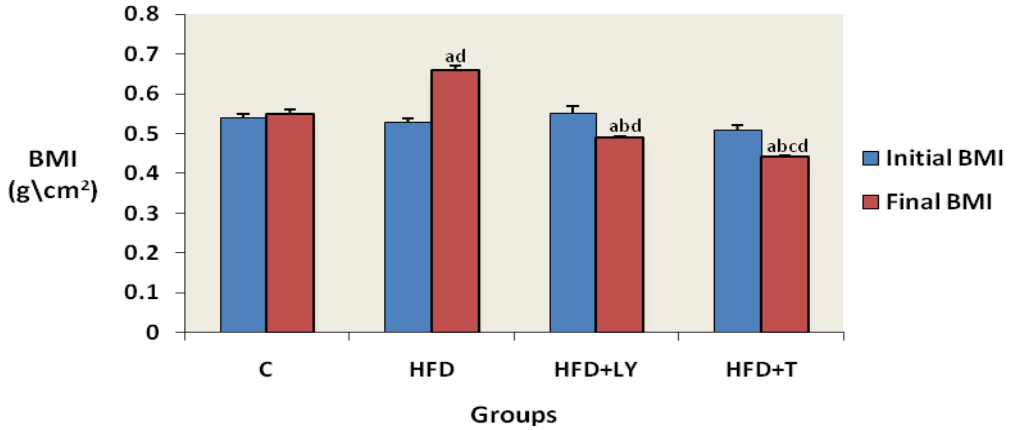

Figure (٢): The BMI (g\cm^{٢}) in the different studied groups. HFD: high fat diet, HFD+T: HFD+Tramadol treated group, BMI: Body mass index. a: Significant from control group (C), b: Significant from HFD group, c: Significant from HFD+LY (HFD+LY٢٥٥٥٨٢ treated group), d: Final value significant from its corresponding initial value, $P< ٠.٠٥$. Data are expressed as mean ± S.E.M. of ٨ rats in each group.

Table (١): Changes in the food intake during the induction weeks of obesity in the different studied groups.

Groups / Food intake (g/d)	C	HFD	HFD+LY	HFD+T
After ١ week (Initial week)	٢٠.٥ ± ٠.٩	٢٤.٥ ± ١.٤^{a}	٢٠.٨ ± ١	٢٩.١ ± ١.١^{abc}
After ٢ weeks	١٨.١ ± ١	٢٤.٣ ± ١.٣^{a}	١٩.٤ ± ٠.٩^{b}	١٠.٩٥ ± ٠.٧^{abc}
After ٣ weeks	١٩.١ ± ٠.٣	٢٠.٥ ± ٠.٧	١٨.١ ± ٠.٥^{b}	٦.٨ ± ٠.٣^{abc}
After ٤ weeks (Final week)	١٩.٤ ± ٠.٢٣	١٨ ± ٠.٥^{ad}	١٦.٥ ± ٠.٢^{abd}	٥.٢ ± ٠.١^{abcd}

Data are expressed as mean ± S.E.M of ٨ rats in each group. HFD+T: HFD+Tramadol treated group. a: Significant from control group (C), b: Significant from HFD group, c: Significant from HFD+LY (HFD+LY٢٥٥٥٨٢ treated group), d: Final week significant from its corresponding initial week, $P< ٠.٠٥$.

Table (٢): Serum levels of lipid profile, glucose, insulin and leptin at the end of the induction weeks of obesity in the different studied groups.

Parameters / Groups		C	HFD	HFD+LY	HFD+T
Lipid profile	TC (mg/dl)	٦٩.٢ ± ١.٢	٨٩.٣ ± ٢ ^{a}	٦٠.٥ ± ٢.٦^{ab}	٩٩± ٢.٨^{abc}
	TGs (mg/dl)	٤٤.٥ ± ١.٧	٧٤.٨ ± ٣.٢ ^{a}	٣٤.٨ ± ٢.٣^{ab}	٨٣.٥± ٣^{ac}
	HDL-c (mg/dl)	٢٥.٢ ± ١.٩	١٧.١ ± ١.١^{a}	٣٢.٥ ± ٢.٢^{ab}	١٨.١± ١.٧ ^{ac}
	LDL-c (mg/dl)	٣٤.٩ ± ١.٨	٥٧.٤ ± ٢.٧ ^{a}	٢٠.٧ ± ١.٧^{ab}	٦٤.٢ ± ٢.٤ ^{ac}
Serum glucose (mg/dl)		٥٨.٩ ± ٢.٤	٩٨.٩ ± ٤.١ ^{a}	٦٠.٩ ± ٢.٩^{b}	٩٧.٦ ± ٥.٢ ^{ac}
Serum insulin (mIU/l)		٣.١ ± ٠.١	٤.٣ ± ٠.٢^{a}	٣.٢ ± ٠.٢^{b}	٤.٦± ٠.٢ ^{ac}
Serum leptin (ng/ml)		٣.٩ ± ٠.٣	٨.٥ ± ٠.٢^{a}	٥.١ ± ٠.٥^{b}	٣ ± ٠.٢^{abc}

Data are expressed as mean ± S.E.M of ٨ rats in each group. HFD+T: HFD+Tramadol treated group, TC: Total cholesterol, TGs: Triglycerides, HDL-c: High Density Lipoprotein-cholesterol, LDL-c: Low Density Lipoprotein-cholesterol. a: Significant from control group (C), b: Significant from HFD group, c: Significant from HFD+LY (HFD+LY٢٥٥٥٨٢ treated group), $P< ٠.٠٥$.

Discussion

Obesity is a worldwide health threat challenge[١٧]. Therefore, it is necessary to actively explore approaches for this problem. In this study, we are focusing on the effect of an opioid agonist and antagonist against HFD induced obesity.

In the present study, the result clearly demonstrated that the final body weight of the control group significantly increased as compared with its initial body weight, in spite of the fact that BMI and food intake weren't changed. This could be explained by

a natural body growth with no excess fat deposition since the BMI did not significantly change. This agrees with the results reported by Ble-Castillo et al.,[١٨].

In the present study, obesity was induced in male albino rats by using a HFD formula for ٤ weeks. The weight gained by rats fed HFD formula, was significantly more than that gained by those fed the normal diet after ٤ weeks from the start of experiment as proved by significantly higher final BMI and body weight from the second week up to the end of the study. Many workers were able to induce obesity in rats using different formulas of high fat diets[١٩].

This weight gain could be explained by the fact that HFDs are characterized by a high palatability that is often considered to increase the energy intake and also promote hyperphagia[٢٠] as proved in our study by a significant higher amount of initial food intake as compared with control group. Other factors that may contribute to obesity induced by HFD include the overcon-sumption of high caloric diet and poorly satiating properties of the HFDs[٢١].

Another possible mechanism of increased body weight is the increased lipogenesis and decreased lipolysis which was evidenced in the present work by a significant increase in BMI. These findings are consistent with Kumar et al.,[٢٢]. In addition, HFD could have caused an attenuation of the vagal afferent function which express receptors for many of the regulatory peptides and molecules released from the intestinal wall, pancreas, and adipocytes that influence GI function, glucose homeostasis, and regulate food intake and body weight. The mechanism(s) leading to this attenuation is not clear but it may be due to an altered balance in expression of anorexigenic and orexigenic peptides and receptors, leading to dysregulation of intestinal feedback control of GI function and food intake[٢٣].

Finally, preference for certain foods, including fats, are a complex behavior regulated by: (a) homeostatic mechanisms, which serve to maintain energy balance, and (b) reward-related mechanisms, which process the hedonic properties of food independently of energy status[٢٤] and this was mediated by the brain opioid systems. This reward effect was supported by Kraft et al.,[٢٥] who found that obesity caused by increasing the preference for a HFD was attributed to the higher expression in the hypothalamus of μ-opioid receptors.

In the final week of the present study, the result clearly demonstrated that the body weight of HFD group still significantly increased in spite of the decrease in the final food intake as compared with control group and its initial values. This could be attributed to the peripheral inhibitory effects of fat on the gastrointestinal motility and stimulation of anorexic GI hormones including cholecystokinin, peptide YY and glucagon-like peptide-١, as well as the suppression of ghrelin which is the only GI orexigenic hormone[٢٦].

In addition, HFD could decrease food intake through central mechanisms mediated by the increase in the leptin level as reported in our study and others[٢٧]. It enters the brain arcuate nucleus (ARC) where it induces/ represses a network of important neuropeptide regula-tors of energy intake and expenditure[٢٨]. However, leptin failed to decrease body weight inspite of its central inhibitory effect on food intake, as leptin alone couldn't fight adiposity in the presence of continuous HFD consumption with higher caloric value as compared with the standard diet[٢٩].

Finally, HFD intake is associated with excessive circulating free fatty acids and glucose, aggravating insulin resistance and increasing lipolysis and insulin secretion [٣٠]. Hyperinsulinemia synergistically acts centrally with hyperleptinemia (Leptin-insulin lipostat) to decrease food intake as these two hormones reach the hypothalamus and activate specific "catabolic" neuro-endocrine circuits, which inhibit food intake[٣١]. This indicated that the central mechanism for insulin is still working in spite of the development of peripheral insulin resistance marked by the associated hyperglycemia. Hyperglycemia could also induce satiety by a peripheral action through

stimulating afferent vagal fibers[٣٢] and additionally induced a state of dyslipidemia *via* stimulation of *de novo* hepatic lipogenesis[٣٣] as observed in our study.

In the present study, the rats that consumed the HFD with injection of LY showed a significant lower amount of final food intake with correlated decrease in final body weight and BMI as compared with control and HFD groups. This could be attributed to inhibition of μ-opioid receptor signaling as reported by Kraft et al.,[٣٥]. Additionally, our result demonstrated that the final body weight significantly increased as compared with its initial value and this could be explained by a natural body growth with no further fat deposition since the final BMI significantly decreased as compared with its initial value. This effect could be secondary to improved glucose tolerance and insulin sensitivity as observed in the present results by the significant lowering of blood glucose. From the above mentioned data, opioids appear to exert their effect on food intake predominantly within the central nervous system, although peripheral effects on taste and gastrointestinal motility play a minor role[٨].

The decreased blood glucose level with LY administration during induction of obesity in the present study could be explained by the increase in insulin sensitivity caused by reduction in body adiposity[٣٤]. Furthermore, it could be mediated through blocking or reversal of the opioid hyperglycemic effects. Such hyperglycemic effects of opioids are in accordance with Mysels and Sullivan[٣٥] who reported that opioid administration caused impairment in key enzymes related to glucose metabolism; the glycolytic activity of hexokinase and phosphofructokinase-١ activity was diminished, leading to less breakdown of plasma glucose, while, the gluconeogenic activity of glucose-٦-phosphatase and fructose-١,٦-biphosphatase was increased, leading to increased production of plasma glucose and a metabolic state similar to non-insulin-dependent diabetes. In addition, opioids were found to induce adrenal excitation through $\alpha_{٢}$-adrenoceptors and caused the subsequent changes in the liver function[٣٦].

The data of the present study clearly demonstrated that administration of opioid antagonist; LY during induction of obesity caused a significant decrease in insulin level. This could be achieved directly by blocking parasympathetic stimulation of insulin release[٣٧], or secondary to the decreased blood glucose level; the main direct stimulant of beta cells[٣٨]. Additionally, LY decreased insulin level indirectly by reduction in the body fat, with a consequent increased insulin sensitivity in accordance with Paspala et al.,[٣١]. Because serum leptin was positively and strongly correlated with BMI[٣٩], and since LY administration decreased the BMI in the present work, so leptin level significantly decreased.

The relationship between insulin resistance, hyperglycemia and dyslipidemia is mutual and is a cause and effect response[٤٠,٤١]. Insulin decreases adipose tissue lipolysis and improves plasma lipid profile[٤٢]. LY; in the present work decreased blood glucose level and improved insulin sensitivity, hence the observed correction of the dyslipidemic effect produced by the HFD.

In the present study, the treatment with tramadol (opioid agonist) with HFD feeding resulted in marked decrease in the food intake, body weight and BMI and this may be a cause for decreased leptin level that was observed in our study because serum leptin was strongly correlated with BMI. These results are in agreement with Anghel et al.,[٤٣] who reported a decrease in the food intake coupled with marked weight loss after opioid agonist administration. They reported that the long-term opioid agonist treatment act centrally by decreasing hypothalamic neuropeptide Y, agouti-related protein, and cocaine and amphetamine regulated transcript expression which is considered potent orexigenic peptides. This supports the anorexic effect of morphine, but disagrees with Verbeek et al.,[٤٤] who reported that the administration of opioid agonist increased the food intake and body weight in rats by enhancing the rewarding properties of food.

In the present study, treatment with tramadol failed to produce any significant change in

the serum levels of TGs, HDL-c, LDL-c, glucose and insulin as compared with HFD group. This implies that tramadol could not significantly modulate the peripheral dislipidemic effects of HFD administration. In accordance with these results and by using other opioid agonist without HFD feeding Mami et al.,[٤٥] suggested a dyslipidemic effect of opioid agonist possibly due to direct lipolytic effect of opioid agonist on isolated epididymal fat pads of rats that lead to increased lipase activity and increase in the rate of fatty acid release. Additionally, Vahidi et al.,[٣٦] reported that the opioid agonist potently stimulates the secretion of both glucagon and insulin; therefore, the direct action of the opiate is increased peripherally on the islet cells of the pancreas.

In conclusion and according to our results, the opioid antagonist; LY significantly decreased the food intake, body weight and BMI, in addition to the improvement of HFD related metabolic abnormalities in the form of dyslipidemia and insulin resistance during the dynamic phase of obesity development as it acts centrally and peri-pherally. The central effect of opiate receptors to enhance food intake appears to be dominating hence, the satiating effect of the opioid antagonist was more significant. In addition, treatment with the opioid agonist; tramadol also caused marked decrease in the food intake, body weight, and BMI, but without any significant effect on HFD induced dyslipidemia. The fact that both opioid agonist and antagonist decreased food intake, body weight and body mass index with variable metabolic effects reflects the discrepancy between the roles of different opioid receptors subtypes, their locality; whether centrally or peripherally mediated on regulation of food intake and explains the greater controverse in research results and the difficulty of non selective opioid receptor agonists or antagonists in obesity management.

However, the opioid receptor antagonist; LY٢٥٥٥٨٢ showed better anti-obesity, and anti-dislipidemic effects in the present work. Finally, future studies, using selective opioid receptor antagonists in obese rats either alone or in combination with other anti-obesity measures are required to establish an ideal model of obesity management.

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