Assess the Effect of Liraglutide on Serum Proinflammatory And Antiinflammatory Adipocytokines In Insulin Resistance Induced Male Wistar Rats

Furqan Mohammed Abdulellah[1]   Mustafa Ghazi Al-Abbassi[1]   Dalia Abd Alkader Shakur[1]   Ahmed Mohammed Khalaf[2]

[1]*Department of Pharmacology and Toxicology, College Of Pharmacy, Mustansiriya University/Iraq.*
[2]*Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University/ Amman-Jordan.*

Abstract

Objective:
The current study was designed to assess the effect of liraglutide on serum adipokines of adult male wistar rats presented with IR that induced by feeding HFD. Where high fat diet pellets (HFD) induce obesity associated with insulin resistance (IR) and low-grade inflammation of adipose tissue. Liraglutide is a glucagon like peptide analogue has anti-inflammatory effect that produce significant reduction of serum level of pro-inflammatory adipokines and hence reduce body weight.

Materials and methods:
Thirty-six adult male wistar rats (weighing 200-220gm) were utilized in this study. They divided into 2groups: group A which consist of 12 rats receiving normal pellets and HFD group which has 24 rats feeding HFD pellets. Animals fed HFD pellets for 8 weeks to develop IR were divided into 2 groups:

- Group B feeding HFD pellets for 8 weeks then administered 0.5ml/kg normal saline intraperitoneal for four weeks.
- Group C feeding HFD pellets for 8 weeks then received 600µg/kg/day intraperitoneal liraglutide +0.5ml/kg normal saline four weeks.

Results:
HFD pellets produced a significant* increase in blood glucose and body weight of HFD group. Liraglutide showed a significant* increase in serum level of anti-inflammatory adipokines of group C. It also produces a significant* decrease in serum level of pro-inflammatory cytokines of group C when compared with group B and control group.

Conclusions:
Liraglutide has anti-inflammatory effects that significantly* elevate serum level of anti-inflammatory cytokines and significantly* reduce serum level of pro-inflammatory cytokines.

Keywords: Insulin resistance; Obesity; High fat diet (HFD); Liraglutide; Subfatin; Adipolin.

INTRODUCTION

Diabetes mellitus (DM):

Diabetes mellitus is a chronic unorganized condition of hyperglycemia due to impaired insulin secretion, insulin action or both[1]. Different pathogenic causes are ensued in diabetes development, these ranging from autoimmune damage of pancreatic β-cells which cause insulin deficiency to defects that develop resistance to insulin action[2].

Insulin resistance (IR) is the state in which insulin-encouraged glucose uptake is blunted in the insulin sensitive-tissue leading to state of prediabetes and T2DM, IR characterized by hyperglycemia and hyperinsulinemia in the fasting state, elevated glycosylated hemoglobin (HbA1c) level, hyperlipidemia, postprandial hyperglycemia, elevated plasma levels of pro-inflammatory markers and hypoadiponectinemia[3]. IR disturbs liver and skeletal muscle׳s utilization of glucose causing a reduction in glycogen storage and impairs glucose homeostasis causing perturbation of intracellular and extracellular glucose levels, accordingly augmenting insulin exocytosis from pancreatic B-cell leading to hyperinsulinemia[4]. IR is an important pathological issue of metabolic diseases such as hypertension, T2DM, obesity and hyperlipidemia[5].

IR was induced when nutrient storage exceed energy expenditure leading to accumulation of lipid in liver and skeletal muscle (lipotoxicity) inducing cascades that impair insulin signaling [6, 7]. Obesity associated with chronic low-grade tissue inflammation is a major cause of IR; Accumulation of activated macrophages in adipose tissue (AT) with an increased expression of pro-inflammatory genes including a set of cytokines, mainly TNF-α that are secreted markedly from activated macrophages and directly induce IR through action on insulin target cell in confined tissues by a paracrine mechanism[8]. TNF-α signaling triggers intracellular kinases like IκB kinase (IKK) and Jun N-terminal kinase (JNK), which impedes insulin receptor signaling via phosphorylation of serine residue of insulin receptor substrate-1 (IRS-1), as well as initiation of activator protein-1 (AP-1) transcription factors and NF-κB effects feed-forward mechanism by exacerbating pro-inflammatory cytokines production. An excessive level of cytokine secretion leads to leaking out of cytokine from tissue, raising the circulating concentrations and initiating endocrine effects on distant organ system like liver and muscle causing systemic IR[9, 10].

Obesity is worldwide health problems correlated with IR, dyslipidemia, hypertension and hyperglycemia, which are all known as metabolic syndrome (MS) [11]. The most predominant MS is T2DM and is described as defects in secretion of insulin and peripheral IR in the AT, skeletal muscle and the liver [12]. Overweight together with obesity are regarded as the accumulation of excessive body fats particularly abdominal fat which has biological metabolic activity and correlated with immune activation and low-grade systemic inflammation. Activated macrophages and enlarged adipocytes produce pro-inflammatory cytokines and hormones like leptin, which favored the cellular Th-1 type immune response leading to obesity. At later obesity stages the main harmful effects of AT inflammation on systemic glucose tolerance and adipose insulin sensitivity become obvious so reducing the ability of AT to act as a lipid reservoir and its ability to isolate fatty acids far from peripheral tissues, therefore, inflammation of AT may participate in the suppression of systemic insulin sensitivity and glucose tolerance in adipocytes via downregulation of *de novo* insulin lipogenesis and insulin action [13].

Exercise and dietary restriction play an important role in reducing abdominal fat mass thereby decreasing the secretion of pro-inflammatory adipokines [14]. Obesity assessment is done by measuring body mass index (BMI) which is calculated by dividing the weight in kilograms by the square of height in meters. BMI is a poor discriminator of lean mass and body fat, therefore,

we have additional measurements for obesity and fat distribution, like waist circumference (WC) and waist-to-hip ratio (WHR)[15].

Two leptin-sensitive neuronal circuits in the hypothalamic arcuate nucleus (ARC) have an important role in regulation of energy expenditure and food intake: activation of orexigenic neuron which is agouti-related peptide and neuropeptide Y (AgRP/NPY) strongly decreases energy expenditure and increases food intake via paracrine action of AgRP and NPY themselves and by hindrance of GABAergic neurons signaling [16]. On the other hand, increased energy expenditure and reduced food intake ensues with stimulation of anorexigenic neurons which are proopiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART). Therefore, convenience functioning of these neuron subsets is prerequisite for appropriate energy homeostasis. Microglia and astrocytes have a major role in the response of CNS to injury via providing either prolonged neurotoxicity or neuroprotection relying on the kind of the underlying harm. Impaired hypothalamic neuronal function augmenting inflammatory signals via recruitment and stimulation of pro-inflammatory cells in AT and other peripheral tissues. Consequently, associated with leptin resistance and weight gain [17].

AT is initially differentiated into brown adipose tissue (BAT) and white adipose tissue (WAT), while pink and beige AT are also recognized. The former has large cytoplasm and multiple lipid droplets scattered throughout, with abundance of mitochondria that regulate heat production and energy expenditure [18, 19]; sympathetic nervous system innervates BAT, hypothalamus stimulated during cold thus promote sympathetic outflow to BAT thereby stimulating noradrenalin release from efferent sympathetic nerve terminals, then adrenalin bind to β- adrenergic receptor, the activated

receptor has both acute and chronic effect on BAT: acute thermogenesis stimulate fatty acid breaks down, lipolysis, glucose uptake and activation of uncoupling protein-1 (UCP-1) while chronic stimulation lead to increased UCP-1 gene transcription and mitochondrial biogenesis resulting in uncoupling of oxidative phosphorylation and dissipating energy in the form of heat[20]. WAT has been believed initially as metabolically inactive and considered as dormant tissue, WAT now recognized as integral endocrine gland that secrete excess of bioactive polypeptides collectively dubbed adipokines (AdK) that permitting communication with other organs, such AdK includes adiponectin, leptin, visfatin, resistin, interleukin (IL)-6, IL-8, plasminogen activator inhibitor-1 (PAI-1), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor (TNF)-α [18, 19]. A Wide range of biological activities including homeostatic and pathological function are regulated by these AdK [21].

Macrophages are the most important immune cells that regulate AT function, ATM increased in number and change their localization and inflammatory state during obesity. Unlike the distribution of ATM in lean individuals which are distributed throughout AT and limiting inflammatory status, in obese patient ATM localized around dead adipocytes and form so- named crown-like structure (CLS) predisposing to profound pro-inflammatory characteristics [22]. Macrophages can be divided into classically activated macrophages (M1) that induce microbicidal activity, and alternatively activated macrophages (M2) that induce allergic activity; in concerned with insulin action, M2 macrophages maintain insulin sensitivity through secretion of interleukins IL-4 and IL-10, while M1 macrophages induce insulin resistance through releasing pro-inflammatory cytokines like TNF-α [23]. M2:M1 ratio in lean AT is approximately 4:1, in AT of obese patients M2 expanded but not as much as M1; some of M2 remain localized in CLS where they play a role in phagocytosis of dead adipocytes, tissue remodeling and angiogenesis. Adiponectin can induce M2 polarization in mouse

and human AT macrophages by increasing expression of IL-10, adiponectin synthesis is higher in lean AT and inversely correlated with obesity and inflammatory marker levels such as IL-6 and C-reactive protein so induce macrophages to produce more IL-10 [24]. During expansion of AT in obese patients ATM accumulated and inducing tissue remodeling by scavenging dead adipocytes and storing lipids forming unique lipid-laden ATM subpopulation AT foam cells, increased number of lipid foams cells in obese patients indicating systemic inflammation and insulin resistance, with the major source of ATM during chronic obesity is circulating blood monocytes [25].

Adipokines are polypeptide or proteins secreted from AT and play an important role in regulation of energy and vascular homeostasis as well as immunity and pathogenesis of MS [26], AdK act in paracrine, autocrine or endocrine manner to regulate various metabolic functions, AdK act locally or systemically and implicated in regulation of insulin sensitivities in insulin-sensitive organs like liver [27]. AdK are classified into: pro-inflammatory and anti-inflammatory AdK depending on the inflammatory response of AT [28].

Leptin is obesity gene product which is secreted by adipocytes regulating appetite and energy metabolism [29]. Nearly all of immune cell express long form of leptin receptors which initiate intracellular signaling that induce activation of tyrosine kinase JAK2, the latent transcription factor MAPK, STAT3, PI3K and ERK1/2 pathways that induce innate immune responses. Indeed, leptin can directly induces the production of pro-inflammatory cytokines like TNF-α, IL-6, IL-12 and IL-18 [30]. Leptin deficiency decrease sympathetic nervous system activity, reduce the immune response, decrease blood pressure, reducing bone density and delay puberty resulting in infertility [31]. Leptin is an anorexigenic hormone that plays an important role in the regulation of food intake and energy homeostasis, it is produced mainly by adipocytes in a pulsatile fashion. nutritional and hormonal state regulate leptin level, it acts together with ghrelin, cortisol and peptide YY to regulate food intake [32]. Under ordinary states secretion of satiety hormone leptin from adipocytes stimulated by feeding, leptin act on its receptors in hypothalamus center especially ARC neuron (AgRT/POMC) causing appetite suppression. Chronic high fats diet HFD is common cause of disturbing leptin signaling in hypothalamus leading to the state of hyperphagic obesity and leptin resistance [33].

Subfatin, gene array analysis identifies subfatin as a novel AdK fortified by caloric restriction in diet-stimulated obese rats it is also known as Cometin, Metrnl and meteorin. Its importance shown in insulin sensitizing action that may explain its promising role as therapeutic target for IR. its expression is greater in WAT than in BAT of both humans and mice [34, 35]. In WAT the expression of meteorin- like is decreased during caloric restriction, whereas it is significantly increased in AT during differentiation of adipocytes and diet-induced obesity in rats [36].

Adiponectin is an anti-inflammatory adipokine derived from adipocyte and has a number of health-protective roles like anti-inflammatory, anti-atherogenic and insulin sensitizing characteristics. The level of circulating adiponectin reduced in obese patients thereby increasing the risk of MS, T2DM, and cardiovascular diseases [37]. There is inverse relation between adiponectin and obesity proposing that weight gain reduces expression of adiponectin [38].

Adipolin which is also identified as C1q/TNF-related protein-12(CTRP12), this adipokine is expressed by AT mainly from subcutaneous fat and it is recognized as an adipose derived insulin sensitizer. Despite of the unclear correlation between body fat percentage and the level of adipolin in human, entire adipolin levels reduced considerably in diet induced obesity in mice[39]. Adipolin ameliorate insulin sensitivity thereby inhibiting

gluconeogenesis as well as improving glucose uptake by hepatocytes and adipocytes [40].

Ghrelin is a peptide composed of 28 amino acid residues synthesized mainly by stomach, additionally lower amounts detected in pancreas, bowel, placenta, immune system, kidney, testis, lung, pituitary and hypothalamus. Physiologically, ghrelin level increased after weight loss and during fasting, while its secretion is inhibited during feeding[41]. Ghrelin present in two forms in circulation: acylated and desacylated, Acylation is important for appetite stimulation (orexigenic activity), neuroprotection, affect glucose homeostasis, mood, anxiety and stress, inflammation and immunity, memory, learning and olfaction. These diverse effects are induced by ghrelin action on growth hormone secretagogue receptor 1A (GHSR) which is member of transmembrane G –protein coupled receptor, whereas anorexigenic activity exerted by desacyl ghrelin which also regulate other physiological function including cerebral blood vessel proliferation and glucose homeostasis even though a receptor for desacyl-ghrelin has not been recognized[42]. Ghrelin is the solitary orexigenic peptide; energy intake causing plasma ghrelin level fluctuation in lean individuals: plasma ghrelin level increase in cachexia and calorie restriction whereas obese patients (except Prader-Willi syndrome who have more than usual plasma ghrelin level) have lower plasma ghrelin level than lean individuals[43].

Liraglutide is an analogue of acylated human glucagon like peptide-1 (GLP-1) that secreted from distal L-cells of small intestine and promptly destroyed by the effect of dipeptidyl peptidase-4 enzyme (DPP-4), therefore it has very short half-life (1.5-2.5 minute) if given intravenously and must be given by a constant infusion to reach therapeutic significance. In order to cope the short half-life of human GLP-1 liraglutide with 97% amino acid similarity to native GLP-1 is developed. The difference between liraglutide and native GLP-1 is the acylation of lysine residues with hexadecanoyl

glutamyl side chain at the position 26. And a single replacement of lysine by arginine at position 34[44, 45].

Liraglutide improve meal-stimulated insulin secretion so they called incretin mimetic. It is GLP-1 receptor agonist (GLP-1RA) has strong impedance against degradation by DPP-4, so its half-life longer than native GLP-1. GLP-1RA adjust weight loss and glucose control via GLP-1 receptors in the CNS or indirectly through activation of peripheral neurons, ARC of the hypothalamus is the main target of liraglutide, especially the POMC/CART anorexic neuron inducing weight loss. In addition to this direct effect, liraglutide indirectly act through GABAergic neurons and inhibit the orexigenic effect of NPY/AgRP of the ARC inducing weight loss and reduce food intake[46].

This study was designed to assess the effect of liraglutide on serum adipokines of adult male wistar rats presented with insulin resistance that induced by feeding HFD.

MATERIALS AND METHODS

Antiseptic spirit (Areej), ketamin (kepro Holland), liraglutide 1.8mg (Novo-Nordisk), normal saline (pioneer), phosphate buffer saline (Sigma-Aldrich) and xylazine (kepro Holland). As well as a specific sandwich ELISA kits from Mybiosource USA company for detection of serum adiponectin, adipolin, leptin and subfatin using microplate washer (BioTek ELx50) and reader (BioTek ELx800).

METHODS
Animal design

Thirty-six adults male Wistar rats (weighing 200-220gm) were utilized in this study. They were attained and placed in animal house of University of Mustinsiryia/ College of pharmacy, they were received water and ordinary pellets for 10 days for acclimatization, after that they divided into 2groups: normal diet group which consist of 12 rats receiving normal pellets and HFD group which has 24 rats feeding HFD pellets (HFD pellet composed of 59%fats, 14% protein and 23% carbohydrates)[52]. Animals fed HFD pellets for 8 weeks to develop IR were divided into 2 groups as illustrated in table 1.

Animals were kept in plastic cages each with dimension (20x25x35 cm) that harbor two rats, animals within same cage were discriminated by back fur marketing using waterproof black marker. Before the beginning of the study protocol, the animals were placed for 10 days under controlled condition of room temperature (21 ± 1^0C) and light stream of 12 hours light/ 12 hours dark cycles. It is worth mentioning that animal house was supplied with an air vacuuming machine. Foods (ordinary and HFD pellets) and water were attainable easily to animals. Animals study design started in 1/11/2017 and ended in 1/2/2018. Ethics committee of the College of pharmacy/ Mustansiriyah University was given their approval to begin the study.

Measurements of body weight and blood glucose

Initial body weight measurements were taken on the first day of the animal's arrival to the animal house, after acclimatization time, every two weeks and at the end of the 8th week of HFD feeding period. Fasting blood glucose (FBG) were measured for all animals immediately after their arrival to animal house, after acclimatization time, every two weeks, at the end of the 8th week of HFD feeding period and every two weeks for group C during liraglutide administration. By making incision in the tail as illustrated by Fluttert *et al*. 2000 [53]. FBG levels of conscious rats were measured by using glucometer (Accu-Chek Go, Roche) This procedure is carried in conscious rats, using suitable manual holder, the rat was held in horizontal position with its tail dragged and swabbed with antiseptic spirit, after few seconds scratching the tail ̓s vein with Accu-check tip pen lancets then placed drop of blood on the strip that positioned in Accu-check glucometer to measure FBG every two weeks. Finely the weight of each rat in HFD group measured every two weeks to check any increment in the weight and recording the result in tabling form [54, 55]. Blood glucose level must be more than 260mg/dl to indicate development of IR[56]

Treatment administration

Liraglutide (victoza®) was administered intraperitoneal (600µg/kg daily dose) freshly dissolved in Normal saline 0.5ml/kg for four weeks, treatment was administered at the end of the 8th week of chowing HFD [57, 58].

Animals sacrifice and serum sample preparation

At the end of the 12th week, the animals were fasted for 3 hours then anesthetized by intramuscular administration of 50 mg/kg ketamine and 5 mg/kg xylazine[59]. blood collected by syringe from animals (~8 ml) by cardiac puncture (Fig. 1) and the anesthetized animals euthanized by decapitation[60]. The collected blood poured into serum separator tube then blood allowed to clot at room temperature for 2 hours, after that centrifuging for 15 minutes at ~ 1000 x g (or 3000rpm) and 4°C, take out the serum and aliquot in Eppendorf tubes and store samples in deep freezer at (-20 °C or -80 °C) for detection of insulin, leptin, subfatin,acylated ghrelin, adipolin and adiponectin levels; avoid freeze / thaw cycles because it is detrimental.

Statistical analysis

The statistical packages for social sciences (SPSS version 16) used for data analysis [61]. Descriptive data analysis will have expressed as mean ± standard error of mean (SEM), **One-Way ANOVA** test used to differentiate between more than two independent means of all studied parameters of the three groups, followed by Tukey test. **Paired T-test** was used to compare between means of initial and final body weight and blood glucose of the same group [62]. Statistical significant difference was considered when p less than 0.05.

Table 1: Animal grouping and treatment duration

Group	No.	Treatment	Duration
A (control)	12	Receiving normal diet pellets for 8 weeks then normal diet pellets + 0.5ml/kg normal saline intraperitoneal four weeks.	Twelve weeks
B (Insulin resistance)	12	Feeding HFD pellets for 8 weeks then administered 0.5ml/kg normal saline intraperitoneal for four weeks	Twelve weeks
C (treatment)	12	Feeding HFD pellets for 8 weeks then received 600µg/kg/day intraperitoneal liraglutide +0.5ml/kg normal saline four weeks.	Twelve weeks

Table 2: Changes in body weight (g). Data expressed as mean ±SEM.

Group	Initial weight (g)	Final weight (g)	Changes in weigh (g)
Group A	241 ±4.431	242.17 ±4.103[c]	1.167 ±0.588
Group B	244.67±4.609	503.75±2.147 *	295.083±5.996 [a]
Group C	506.25±2.086	306.67±1.703 *	-199.583±2.743[b]

*Significant difference of paired T-test when compared mean of initial and final weight.
[a] Significantly different when compared with group A at $p < 0.05$.
[b] Significantly different when compared with group B at $p < 0.05$.
[c] non-significant difference when compare mean of group C with group A at $p < 0.05$.

Table 3: Changes of blood glucose in mg/dl. Data expressed as mean ±SEM.

Group	Initial blood glucose mg/dl	Final blood glucose mg/dl	Changes in blood glucose mg/dl
Group A	108.92 ±1.018	111.75 ±1.023[c]	2.083 ±0.933
Group B	114.83 ±1.043	264.85 ±3.031*	149.8 ±3.434 [a]
Group C	264.58 ±1.790	132.83 ±1.127 *	-131.750±1.393[b]

.*Significant difference of paired T-test when compared mean of initial and final weight.
[a] Significantly different when compared with group A at $p < 0.05$.
[b] Significantly different when compared with group B at $p < 0.05$.
[c] non-significant difference when compare mean of group C with group A at $p < 0.05$.

Table 4: Mean ±SEM of pro-inflammatory cytokines and HbA1c % of all studied groups.

Mean	Mean ±SEM		
Groups	Insulin	Leptin	Subfatin
Group A	0.849 ±+0.0205[c]	5.485 ±0.118 [c]	32.291±0.541*[a]
Group B	1.246±0.0154*[a]	8.895 ±0.204*[a]	42.028±0.697*[a]
Group C	0.905 ±0.046*[b]	6.800 ±0.270*[b]	34.202 ±0.491*[b]

Data represented as mean ±SEM.
*Significant difference (p <0.05) of One Way ANOVA test.
[a] Significant difference of group B when compared with group A at p <0.05.
[b] Significant difference of group C when compared with group B at p <0.05.
[c] non-significant difference of group C when compared with group A at p <0.05.

Table 5: Mean ±SEM of anti-inflammatory cytokines of all studied groups.

Mean	Mean ±SEM		
Groups	Acylated ghrelin	Adipolin	Adiponectin
Group A	133.767 ±5.896[c]	810.36 ±5.301[c]	5.075 ±0.163 [c]
Group B	57.842 ±1.785*[a]	688.28 ±9.165*[a]	3.050 ±0.063*[a]
Group C	122.687 ±1.310*[b]	761.43 ±9.795*[b]	4.672 ±0.116*[b]

Data represented as mean ±SEM.
*Significant difference (p <0.05) of One Way ANOVA test.
[a] Significant difference of group B when compared with group A at p <0.05.
[b] Significant difference of group C when compared with group B at p <0.05.
[c] non-significant difference of group C when compared with group A at p <0.05.

Fig. 1: Cardiac puncture and blood collection for serum preparation.

RESULTS

Result of this study represented as mean ±SEM and revealed a significant* increase in the mean of blood glucose and body weight as well as serum insulin, leptin and subfatin of group B as compared with group A, and significant* decrease in the mean of blood glucose and body weight in addition to serum level of these pro-inflammatory cytokines of group C after daily liraglutide administration for four weeks when compared with group B (table 2, table 3, table 4 and table 5), (fig 2 A and B), (fig 3A,B and C) respectively. While liraglutide administration showed a significant* increase in serum level of acylated ghrelin, adipolin and adiponectin of group C when compared with group B which significantly* reduced after receiving HFD pellets (fig 4 A, B and C) respectively.
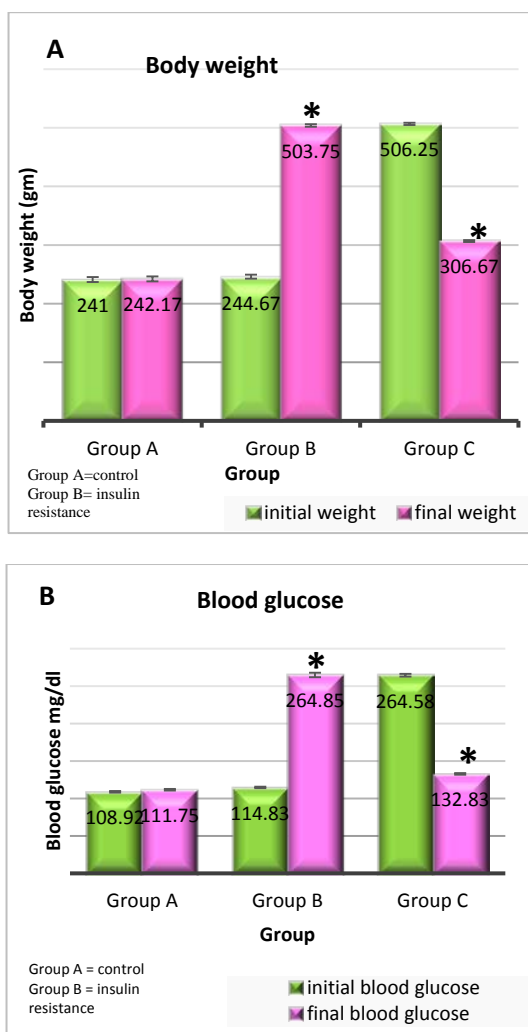

Fig. 2: A- Bar chart show the changes of body weight and **B-** Bar chart show the changes of blood glucose of group B and group C as compared with group A before and after HFD and liraglutide administration, * which represent the significant difference.

DISCUSSION

Body weight changes and obesity

Increase body weight and obesity is a major cause of metabolic syndrome and worldwide health problems, while inflammation is the main contributor of developing obesity-induced insulin resistance due to the role of AT resident macrophages and other pro-inflammatory cells like Th1 CD4 T cells, neutrophils, CD8 T cells, mast cells and B- cells that also found in AT. Anti-inflammatory cells counteract these pro-inflammatory cells that also contained in AT and include eosinophils and Th2 CD4 T

cells; therefor obesity result from AT inflammation that represented by shifting of the homeostatic balance of these two pathways towards pro-inflammatory status[63]. HFD cause increasing energy intake and energy stores in WAT, so lead to obesity. HFD is consistent with insulin resistance in BAT and reduced glucose uptake in the liver, WAT and muscle; this will illustrate the increase in body weight of group B and group C (insulin resistance and treatment groups respectively) that fed HFD for eight weeks and showed significant* increase in body weight and in developing insulin resistance when compared with group A (control group)[64].
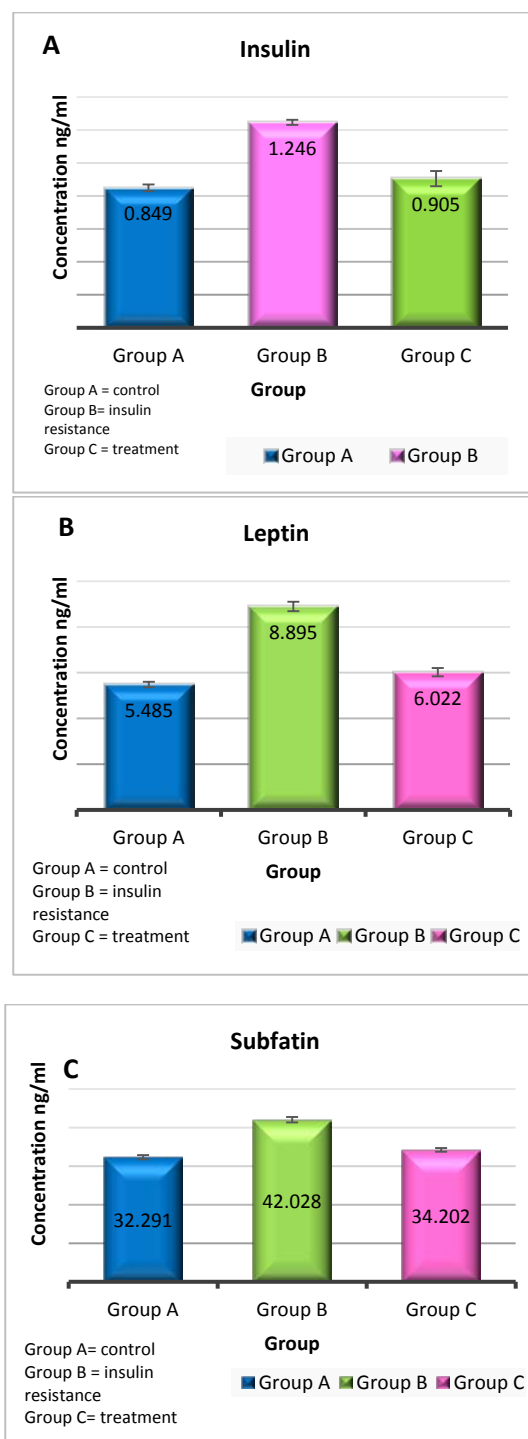

Fig. 3: A- Insulin, **B-** Leptin and **C-** Subfatin mean ±SEM of all studied groups.

Acylated ghrelin

Group A = control
Group B = insulin resistance
Group C = treatment

Adipolin

Group A = control
Group B = insulin resistance
Group C = treatment
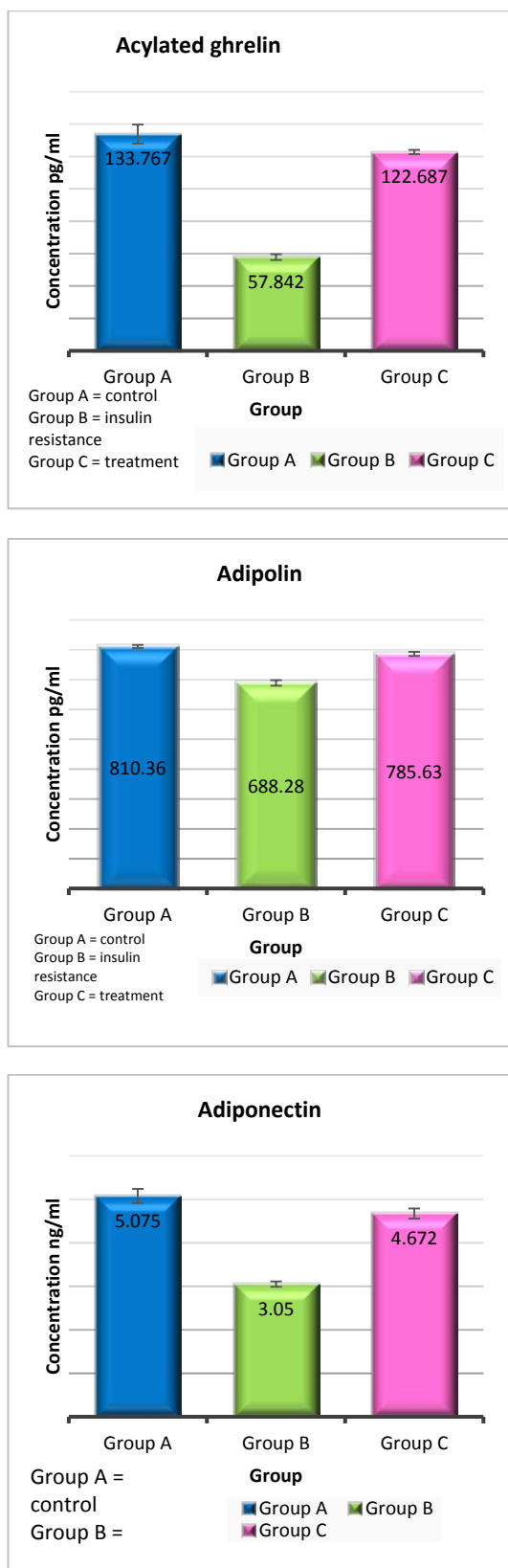
Adiponectin

Group A = control
Group B =

Fig 4: A- Acylated ghrelin, **B-** Adipolin and **C-** adiponectin level of all studied groups represented as mean ±SEM.

Liraglutide which is GLP-1 RA supress food intake centrally via its action on ARC that significantly increase CART gene expression in the same time liraglutide inhibits the routine activation of NPY/AgRP; Inhibition of routine stimulation of NPY/AgRP cause inhibition of food intake and continued lose in

body weight. Additional mechanisms by which liraglutide reduce food intake and hence body weight by delay gastric emptying which participate to their anorexigenic effects [65]. In addition to all previous mechanisms mentioned above, liraglutide activate BAT and stimulate browning of WAT (also known as beige AT) which results in increase energy expenditure[66]. These mechanisms will demonstrate the significant* decrease inbody weight of group C after four weeks of intraperitoneal administration of 600µg/kg liraglutide plus 0.5ml/kg normal saline.

Insulin resistance and blood glucose changes
Elevated blood glucose via increase rates of hepatic gluconeogenesis and failure of insulin to restrain this process these lead to T2D. hepatic glucose production suppressed by insulin action through constriction of fatty acid influx to the liver leading to reduced concentrations of hepatic acetyl Co A and suppressed action of pyruvate carboxylase (PC) which is the beginning step that convert pyruvate to glucose. Excess lipolysis of WAT which mediated by inflammatory cytokines is accountable for raised fasting hepatic glucose production and decreased insulin-mediated inhibition of hepatic glucose production in HFD rodent models linked with hepatic IR and hyperglycemia, figure (4-1)[67]. This agreed with significant* increased blood glucose of group B and group C after feeding HFD for eight weeks.

Liraglutide augments insulin exocytosis, decrease post-prandial glucose, delay gastric emptying and potentiate the satiety response, liraglutide efficacy of reducing blood glucose was approved and this explain the significant* reduction in blood glucose of group C after daily intraperitoneal administration of 600µg/kg diluted with 0.5ml/kg normal saline for four weeks[68].

Pro-inflammatory cytokines
Changes in serum insulin concentration
Significant* raised serum insulin concentration of group B after HFD feeding for eight weeks demonstrate that HFD induce obesity-associated insulin resistance and hyperinsulinemia by inhibition of AMP-activated protein kinase (AMPK) through increase gluconeogenesis and lipogenesis and decreased fatty acid oxidation[69]. While significant* drop of serum insulin level of group C after daily administration of 600µg/kg plus 0.5ml/kg normal saline for four weeks when compared with serum insulin level group B explained the effect of liraglutide on improving insulin sensitivity through activation of AMPK[70]
Changes in serum leptin level
Obesity induced by HFD feeding associated with low grade tissue inflammation in the brain and peripheral tissues and have important role in diet induced adiposity and leptin resistance through increase production and secretion of pro-inflammatory cytokines that disturb hypothalamic leptin signalling via activation of TLR4 dependent IKKβ/NFκB pathway[71], this will correspond with significant* increase in serum leptin level of group B after feeding HFD for twelve weeks when compared with control group.
Previous studies show that liraglutide reverse leptin resistance and reduce serum leptin level in obese mice and non-alcoholic liver steatosis patients through down regulation of the suppressor of cytokines-3 (SOCS3) by inhibiting JAK/STAT pathway[72]. The result of this study was consistent with liraglutide improving insulin sensitivity and leptin sensitivity by reversing leptin resistance and this well demonstrated by significant*
decrease in serum leptin level of group C after daily administration of liraglutide for four weeks when compared with group B.

Changes in serum subfatin level

Subfatin is a novel meteorin-like protein that has pro-inflammatory effect highly expressed in white adipose tissue lead to excessive energy accumulation and M2 polarization[73], which is consistent with this study that showed a significant* increase in the mean of serum subfatin of group B after feeding HFD when compared with the mean of serum subfatin of group A.

Liraglutide daily administration showed a markedly decrease in the mean of serum subfatin level of group C after four weeks of receiving 600μg/kg of liraglutide plus 0.5ml/kg normal saline, despite there is no previous study of liraglutide on subfatin level but the result of this study might consistent with the anti-inflammatory effect of liraglutide.

Changes of serum anti-inflammatory cytokines
Changes of acylated ghrelin level

Acylated ghrelin concentration negatively related with HFD induce obesity associated with low grade tissue inflammation while fasting condition stimulate acylated ghrelin secretion from the stomach that regulate feeding and adiposity. Acylated ghrelin act centrally on GHSR that mediate feeding behaviour and adiposity (orexigenic effect)[74]. This study investigates that HFD significantly* decrease serum level of acylated ghrelin of group B that depend on HFD for twelve weeks this demonstrate the orexigenic effect of acylated ghrelin and its compensatory mechanism in attempt to regulate energy homeostasis.

Acylated ghrelin act directly on pancreatic B-cells and improve glucose dependent insulin sensitivity, liraglutide increase serum level of ghrelin independent on GLP-1 level and mediate its body weight reducing effect through regulation of serum ghrelin concentration[75]. This consistent with the result of this study that shown a significant* increase in serum level of acylated ghrelin of group C after daily administration of 600μg/kg of liraglutide plus 0.5ml/kg normal saline for four weeks when compared with group B.

Changes of adipolin level

The anti-inflammatory and insulin sensitizing effect of adipolin as well as its role in browning of adipose tissue[76] was revealed in this study through a significant* decrease in the mean of serum adipolin of group B after feeding HFD when compared with the mean of adipolin of group A.

Despite of no previous study demonstrate the effect of liraglutide on adipolin, this study showed that liraglutide significantly elevates serum adipolin level of group C after daily administration of liraglutide for four weeks and this will agree with its anti-inflammatory and insulin sensitizing effect.

Changes of serum adiponectin concentration

Adiponectin has a reciprocal relation with body weight and insulin sensitivity, that is mean increase body weight will lower adiponectin level as well as induce insulin resistance and MS[77]. This study reveals that HFD feeding significantly* lower serum adiponectin level of group B after twelve weeks of receiving HFD pellets when compared with group A.

Liraglutide significantly* increase serum level of adiponectin of group C after daily administration of liraglutide 600μg/kg diluted with 0.5 ml/kg normal saline when compared with group B that fed HFD in this study, this explain the anti-inflammatory effect of liraglutide through improving level of adiponectin when body weight decreased[78].

CONCLUSIONS

According to the results of this study, we can conclude that:

- Hight fat diet pellets significantly* increase body weight and induce insulin resistance of group B during twelve weeks when compared with group A that receive ordinary pellets.
- Liraglutide significantly* lower blood glucose and reduce body weight. The effect of liraglutide on adipokines level

may explain the role of GLP-1 receptors in the ARC and its contribution in appetite, browning of adipose tissue and surplus energy expenditure. Therefore, liraglutide can be used to treat obesity and improve insulin sensitivity as well as reduce adipose tissue inflammation by mechanisms differ from that of glucose lowering effect which used for T2DM.

REFERENCES

1. Tiwari BK, Pandey KB, Abidi A, Rizvi SI. Markers of oxidative stress during diabetes mellitus. J of Biomarkers. 2013;2013(1):1-8.
2. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes care. 2014;37(1):S81-S90.
3. Ye J. Mechanisms of insulin resistance in obesity. Frontiers of medicine. 2013;7(1):14-24.
4. Abu MN, Samat S, Kamarapani N, Nor Hussein F, Wan Ismail WI, Hassan HF. Tinospora crispa ameliorates insulin resistance induced by high fat diet in Wistar rats. Evidence-Based Complementary and Alternative Medicine. 2015;2015(1):1-6.
5. Qi J, Yang B, Ren C, Fu J, Zhang J. Swimming Exercise Alleviated Insulin Resistance by Regulating Tripartite Motif Family Protein 72 Expression and AKT Signal Pathway in Sprague-Dawley Rats Fed with High-Fat Diet. J of diabetes research. 2016;2016(1):1-10.
6. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. The J of clinical investigation. 2016;126(1):12.
7. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell. 2012;148(5):852-71.
8. Krause M, Heck TG, Bittencourt A, Scomazzon SP, Newsholme P, Curi R, et al. The chaperone balance hypothesis: the importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management. Mediators of inflammation. 2015;2015(1):1-12.
9. Johnson AM, Olefsky JM. The origins and drivers of insulin resistance. Cell. 2013;152(4):673-84.
10. Odegaard JI, Chawla A. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. Sci. 2013;339(6116):172-7.
11. Wajchenberg BL, Cohen RV. Adipose tissue and type 2 diabetes mellitus. Adipose Tissue and Adipokines in Health and Disease. 2014: Springer; 2014. p. 235-48.
12. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes research and clinical practice. 2014;105(2):141-50.
13. Pedersen DJ, Guilherme A, Danai LV, Heyda L, Matevossian A, Cohen J, et al. A major role of insulin in promoting obesity-associated adipose tissue inflammation. Molecular metabolism. 2015;4(7):507-18.
14. Strasser B. Immune-mediated inflammation as a driver of obesity and comorbid conditions. Obesity. 2017;25(6):987-8.
15. Coutinho T, Goel K, De Sá DC, Carter RE, Hodge DO, Kragelund C, et al. Combining body mass index with measures of central obesity in the assessment of mortality in subjects with coronary disease: role of "normal weight central obesity". J of the American College of Cardiology. 2013;61(5):553-60.
16. Kamitakahara A, Bouyer K, Wang CH, Simerly R. A critical period for the trophic actions of leptin on AgRP neurons in the arcuate nucleus of the hypothalamus. J of Comparative Neurology. 2018;526(1):133-45.
17. Thaler JP, Guyenet SJ, Dorfman MD, Wisse BE, Schwartz MW. Hypothalamic inflammation: marker or mechanism of obesity pathogenesis of Diabetes. 2013;62(8):2629-34.
18. Khan M, Joseph F. Adipose tissue and adipokines: the association with and application of adipokines in obesity. Scientifica. 2014;2014(1):1-7.
19. Apostolopoulos V, Courten MP, Stojanovska L, Blatch GL, Tangalakis K, Courten B. The complex immunological and inflammatory network of adipose tissue in obesity. Molecular nutrition & food research. 2016;60(1):43-57.
20. van den Berg SM, van Dam AD, Rensen PC, de Winther MP, Lutgens E. Immune modulation of brown (ing) adipose tissue in obesity. Endocrine reviews. 2016;38(1):46-68.
21. Comstock SS, Hortos K, Kovan B, McCaskey S, Pathak DR, Fenton JI. Adipokines and obesity are associated with colorectal polyps in adult males: a cross-sectional study. PloS one. 2014;9(1):1-9.
22. Boutens L, Stienstra R. Adipose tissue macrophages: going off track during obesity. Diabetologia. 2016;59:879-94.
23. Tateya S, Kim F, Tamori Y. Recent advances in obesity-induced inflammation and insulin resistance. Frontiers in endocrinology. 2013;4(1):1-16.
24. Pirola L, Ferraz JC. Role of pro-and anti-inflammatory phenomena in the physiopathology of type 2 diabetes and obesity. World j of biological chemistry. 2017;8(2):120-8.
25. Pecht T, Haim Y, Bashan N, Shapiro H, Harman-Boehm I, Kirshtein B, et al. Circulating blood monocyte subclasses and lipid-laden adipose tissue macrophages in human obesity. PloS one. 2016;11(7):1-11.

26. Maury E, Brichard S. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Molecular and cellular endocrinology. 2010;314(1):1-16.
27. Li Y, Ding L, Hassan W, Abdelkader D, Shang J. Adipokines and hepatic insulin resistance. J of diabetes Research. 2013;2013(1):1-8.
28. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. Frontiers in endocrinology. 2013;4(1):1-15.
29. Tsai YC, Leu SY, Peng YJ, Lee YM, Hsu CH, Chou SC, et al. Genistein suppresses leptin-induced proliferation and migration of vascular smooth muscle cells and neointima formation. J of cellular and molecular medicine. 2017;21(3):422-31.
30. Mancuso P. The role of adipokines in chronic inflammation. ImmunoTargets and therapy. 2016;5(1):47-56.
31. Cui H, López M, Rahmouni K. The cellular and molecular bases of leptin and ghrelin resistance in obesity. Nature Reviews Endocrinology. 2017;13(6):338-51.
32. Baskaran C, Eddy KT, Miller KK, Meenaghan E, Misra M, Lawson EA. Leptin secretory dynamics and associated disordered eating psychopathology across the weight spectrum. European J of endocrinology. 2016;174(4):503-12.
33. Sai K, Morioka S, Takaesu G, Muthusamy N, Ghashghaei HT, Hanafusa H, et al. TAK1 determines susceptibility to endoplasmic reticulum stress and leptin resistance in the hypothalamus. J Cell Sci. 2016;129(9):1855-65.
34. Zheng S-l, Li Z-y, Song J, Liu J-m, Miao C-y. Metrnl: a secreted protein with new emerging functions. Acta Pharmacologica Sinica. 2016;37(5):571-9.
35. Booth A, Magnuson A, Fouts J, Foster MT. Adipose tissue: an endocrine organ playing a role in metabolic regulation. Hormone molecular biology and clinical investigation. 2016;26(1):25-42.
36. Rodríguez A, Becerril S, Ezquerro S, Mendez-Gimenez L, Frühbeck G. Crosstalk between adipokines and myokines in fat browning. Acta Physiologica. 2017;219(2):362-81.
37. Alomari MA, Khabour OF, Abu OAA. The association between adiponectin and ADIPOQ gene polymorphisms with obesity among young Jordanian women. Genetika. 2016;48(1):363-72.
38. Singh P, Sharma P, Sahakyan KR, Davison DE, Sert-Kuniyoshi FH, Romero-Corral A, et al. Differential effects of leptin on adiponectin expression with weight gain versus obesity. International J of obesity (2005). 2016;40(2):266-74.
39. Lei X, Seldin MM, Little HC, Choy N, Klonisch T, Wong GW. C1q/TNF-related protein 6 (CTRP6) links obesity to adipose tissue inflammation and insulin resistance. J of Biological Chemistry. 2017;2017(1):1-27.
40. Mehrdadi P, Mohammadi RK, Alipoor E, Eshraghian M, Esteghamati A, Hosseinzadeh-Attar M. The Effect of Coenzyme Q10 Supplementation on Circulating Levels of Novel Adipokine Adipolin/CTRP12 in Overweight and Obese Patients with Type 2 Diabetes. Experimental and Clinical Endocrinology & Diabetes. 2017;125(03):156-62.
41. Tash RME, El-Dydamoni OA, Ezzat AA, Badr BMA, Radwan EE, Ahmed MI. Ghrelin and Leptin in Obese Postmenopausal Patients. Clinical Medicine and Diagnostics. 2016;6(3):77-83.
42. Zigman JM, Bouret SG, Andrews ZB. Obesity impairs the action of the neuroendocrine ghrelin system. Trends in Endocrinology & Metabolism. 2016;27(1):54-63.
43. Fernandez G, Cabral A, Cornejo M, De Francesco P, Garcia-Romero G, Reynaldo M, et al. Des-Acyl Ghrelin Directly Targets the Arcuate Nucleus in a Ghrelin-Receptor Independent Manner and Impairs the Orexigenic Effect of Ghrelin. J of neuroendocrinology. 2016;28(2):1-12.
44. Jacobsen LV, Flint A, Olsen AK, Ingwersen SH. Liraglutide in type 2 diabetes mellitus: clinical pharmacokinetics and pharmacodynamics. Clinical pharmacokinetics. 2016;55(6):657-72.
45. Sjöholm Å. Liraglutide therapy for type 2 diabetes: overcoming unmet needs. Pharmaceuticals. 2010;3(3):764-81.
46. Baggio LL, Drucker DJ. Glucagon-like peptide-1 receptors in the brain: controlling food intake and body weight. The J of clinical investigation. 2014;124(10):4223-6.
47. Nuffer WA, Trujillo JM. Liraglutide: a new option for the treatment of obesity. Pharmacotherapy: The J of Human Pharmacology and Drug Therapy. 2015;35(10):926-34.
48. Tonstad S, Rössner S, Rissanen A, Astrup A. Medical management of obesity in Scandinavia 2016. Obesity Medicine. 2016;1(1):38-44.
49. Clements JN, Shealy KM. Liraglutide: an injectable option for the management of obesity. Annals of Pharmacotherapy. 2015;49(8):938-44.
50. Consoli A, Formoso G. Potential side effects to GLP-1 agonists: understanding their safety and tolerability. Expert opinion on drug safety. 2015;14(2):207-18.
51. Chalmer T, Almdal TP, Vilsbøll T, Knop FK. Adverse drug reactions associated with the use of liraglutide in patients with type 2 diabetes–focus on pancreatitis and pancreas cancer. Expert opinion on drug safety. 2015;14(1):171-80.
52. Pei L, Yang J, Du J, Liu H, Ao N, Zhang Y. Downregulation of chemerin and alleviation of endoplasmic reticulum stress by metformin in adipose tissue of rats. Diabetes research and clinical practice. 2012;97(2):267-75.
53. Fluttert M, Dalm S, Oitzl MS. A refined method for sequential blood sampling by tail incision in rats. Laboratory Animals. 2000;34(4):372-8.
54. Samuel IO, Samuel AO, Donatus AE, Stanley E. Dietary Factors in Fasting Blood Glucose Levels and Weight Gain in Female Sprague Dawley Rats. J of Clinical Nutrition & Dietetics. 2017;3(1):1-7.
55. El-Rashedy HA-RH, Mohamed HMW, Khaled A-FRE-S, Mohamed AK, Tamer MA-A, Hanan AA-H, et al. Role of Fat Feeding on the Diabetic Albino Rats. J of Cytology & Histology. 2013.
56. Chen W-P, Wang Y-D, Ma Y, Zhang Z-Y, Hu L-Y, Lin J-L, et al. Danhong Huayu Koufuye combined with metformin attenuated diabetic retinopathy in Zucker diabetic fatty rats. International J of ophthalmology. 2015;8(6):1094-100.
57. Yang J, Ao N, Du J, Wang X, He Y. Protective effect of liraglutide against ER stress in the liver of high-fat diet-induced insulin-resistant rats. Endocrine. 2015;49(1):106-18.
58. Gao H, Zeng Z, Zhang H, Zhou X, Guan L, Deng W, et al. The glucagon-like peptide-1 analogue liraglutide inhibits oxidative stress and inflammatory response in the liver of rats with diet-induced non-alcoholic fatty liver disease. Biological and Pharmaceutical Bulletin. 2015;38(5):694-702.
59. Struck MB, Andrutis KA, Ramirez HE, Battles AH. Effect of a short-term fast on ketamine–xylazine anesthesia in rats. J of the American Association for Laboratory Animal Science. 2011;50(3):344-8.
60. Alizadeh M, Asad MR, Faramarzi M, Afroundeh R. Effect of Eight-Week High Intensity Interval Training on Omentin-1 Gene Expression and Insulin-Resistance in Diabetic Male Rats. Annals of Applied Sport Science. 2017;5(2):29-36.
61. Gupta SK, Dongare S, Mathur R, Mohanty IR, Srivastava S, Mathur S, et al. Genistein ameliorates cardiac inflammation and oxidative stress in streptozotocin-induced diabetic cardiomyopathy in rats. Molecular and cellular biochemistry. 2015;408(1-2):63-72.
62. Kim H-Y. Analysis of variance (ANOVA) comparing means of more than two groups. Restorative dentistry & endodontics. 2014;39(1):74-7.
63. Lee B-C, Lee J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2014;1842(3):446-62.
64. Roberts-Toler C, O'Neill BT, Cypess AM. Diet-induced obesity causes insulin resistance in mouse brown adipose tissue. Obesity. 2015;23(9):1765-70.
65. Ladenheim EE. Liraglutide and obesity: a review of the data so far. Drug design, development and therapy. 2015;9(1):1867-75.
66. Zhu E, Yang Y, Zhang J, Li Y, Li C, Chen L, et al. Liraglutide suppresses obesity and induces brown fat-like phenotype via Soluble Guanylyl Cyclase mediated pathway in vivo and in vitro. Oncotarget. 2016;7(49):81077-89.
67. Perry RJ, Camporez J-PG, Kursawe R, Titchenell PM, Zhang D, Perry CJ, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell. 2015;160(4):745-58.
68. Krasner NM, Ido Y, Ruderman NB, Cacicedo JM. Glucagon-like peptide-1 (GLP-1) analog liraglutide inhibits endothelial cell inflammation through a calcium and AMPK dependent mechanism. Plos one. 2014;9(5):e97554.
69. Saito T, Nishida M, Saito M, Eitsuka T, Nishida H. The Fruit of Acanthopanax Senticosus (Rupr. Et Maxim) Harms, or Goka, Improves Insulin Resistance in High-Fat Diet Fed Obese Mice. Free Radical Biology and Medicine. 2016;100(1):1-33.
70. Jinnouchi H, Sugiyama S, Yoshida A, Hieshima K, Kurinami N, Suzuki T, et al. Liraglutide, a glucagon-like peptide-1 analog, increased insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp examination in patients with uncontrolled type 2 diabetes mellitus. J of diabetes research. 2015;2015(1):1-9.
71. Quarta C, Sánchez-Garrido MA, Tschöp MH, Clemmensen C. Renaissance of leptin for obesity therapy. Diabetologia. 2016;59(5):920-7.
72. Li N, Zhao Y, Yue Y, Chen L, Yao Z, Niu W. Liraglutide ameliorates palmitate-induced endothelial dysfunction through activating AMPK and reversing leptin resistance. Biochemical and biophysical research communications. 2016;478(1):46-52.
73. Li ZY, Zheng SL, Wang P, Xu TY, Guan YF, Zhang YJ, et al. Subfatin is a novel adipokine and unlike Meteorin in adipose and brain expression. CNS neuroscience & therapeutics. 2014;20(4):344-54.
74. Heppner KM, Piechowski CL, Müller A, Ottaway N, Sisley S, Smiley DL, et al. Both acyl and des-acyl ghrelin regulate adiposity and glucose metabolism via central nervous system ghrelin receptors. Diabetes. 2014;63(1):122-31.
75. Rizzo M, Abate N, Chandalia M, Rizvi AA, Giglio RV, Nikolic D, et al. Liraglutide reduces oxidative stress and restores heme oxygenase-1 and ghrelin levels in patients with type 2 diabetes: a prospective pilot study. The J of clinical endocrinology & metabolism. 2015;100(2):603-6.
76. Tan BK, Lewandowski KC, O'Hare JP, Randeva HS. Insulin regulates the novel adipokine adipolin/CTRP12: in vivo and ex vivo effects. J of Endocrinology. 2014;221(1):111-9.
77. Ahn SV, Jung D-H, Yadav D, Kim J-Y, Koh S-B. Relative contribution of obesity and menopause to the association between serum adiponectin and incident metabolic syndrome. Menopause. 2018;25(2):154-9.
78. Ding M, Liu L, Zhang L, Cui Y, Wang M, Wang P, et al. Changes in insulin resistance are related to liraglutide-induced HMW-Adiponectin increase in obese type 2 diabetes. Int J of clinical and experimental med. 2017;10(7):10257-66.