Evaluation of Liraglutide Effect on Serum Adipocytokines of Adults Male Wistar Rats with Insulin Resistance That Induced by High Fat Diet

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Abstract:
Insulin resistance (IR) is the state in which insulin-stimulated 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.
Type 2 diabetes mellitus reported with peripheral insulin resistance (IR) and reduced production of insulin from pancreatic β-cells, IR elevates plasma fatty acids, decreasing glucose transportation to muscles and increased breakdown of fat finally leads to increased glucose production from the liver.
This study was designed to evaluate the effect of liraglutide on serum adipocytokines of adult male rats with insulin resistance that induced by high fat diet, Chronic high fats diet feeding is a common cause of disturbing leptin signaling in hypothalamus leading to the state of hyperphagic obesity and leptin resistance. Liraglutide improve meal-stimulated insulin secretion so it's called incretin mimetic. It is glucagon like peptide-1 receptor agonist that adjust weight loss and glucose control via glucagon like peptide-1 receptors in the central nervous system or indirectly through activation of peripheral neurons.
Current study utilized thirty-six adult male wistar rats (weighing 200-220gm), they were divided into two main groups: normal diet group (group A) that includes 12 rats receiving normal pellets and high fat diet group (group B and C) which has 24 rats feeding high fat diet pellets. Animals fed high fat diet pellets for 8 weeks to induce insulin resistance were divided into two groups: Group B received high fat diet pellets for 8 weeks then administered 0.5ml/kg normal saline intraperitoneal for four weeks. Group C received high fat diet pellets for 8 weeks then received 600μg/kg/day intraperitoneal liraglutide +0.5ml/kg normal saline four weeks along with high fat diet.
High fat diet pellets caused a significant increase in body weight and blood glucose of high fat diet group. Liraglutide revealed a significant elevation in serum level of anti-inflammatory adipokines of group C. It also produces a significant reduction in serum level of pro-inflammatory cytokines of group C when compared with group B and control group.
As conclusion, anti-inflammatory effects of liraglutide significantly elevate serum level of anti-inflammatory cytokines as well as significantly reduce serum level of pro-inflammatory cytokines.

Key words: Insulin resistance, High fat diet, Liraglutide, Adipocytokines.

تقييم تأثير دواء الليراغلوتايد على مستوى الأديبوسافيتوكينات في مصل ذكور جرذان ويستار المصابة بمقاومة الأنسولين المستحثة بتغذية علف عالية الدسم 
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Introduction:
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[1].

Type 2 diabetes mellitus reported with peripheral insulin resistance (IR) and reduced production of insulin from pancreatic β-cells, IR elevates plasma fatty acids, decreasing glucose transportation to muscles and increased breakdown of fat finally leads to increased glucose production from the liver[2].

Explicit genetic mutations are the causative pathological issues of extreme obesity, most of them "obesity genes" are expressed in CNS, revealing that the monitoring of brain metabolism and food intake are essential for energy balance regulation. It is not well understood how the brain orchestrate energy expenditure with food intake to uphold health and stable body weight[3].

الخلاصة:
مقاومة الأنسولين هي الحالة التي يضعف فيها امتصاص الجلوكوز المحفّز بالأنسولين في الأنسجة الحساسة للأنسولين مما يؤدي إلى حالة من مقاومة السكري والسكري من النوع الثاني، مقاومة الأنسولين التي تتميّز بإرتفاع السكر في الدم وفرط أنسيولين الدم في حالة السقاء، وارتفاع مستويه الهيپوغلوتيك، اللفيلزالي (HbA1c) في الدم، ارتفاع السكر في الدم البالغ، وارتفاع مستويات الابيديوسايكتينيات المولدة للالتهابات في البالغ والمصابين بمقاومة الأنسولين من النوع الثاني من داء السكري يتمّ مقارنتها بحبيبات التغذية من المضادة للالتهاب من الطبيعية، وتم تقسيمهم إلى في الجهاز العصبي المركزي أو بشكل غير مباشر من ي يضبط بالأضافة كجم من المحلول الملين طبيعي لمدة أربعة أسابيع ثم تلقت جرذان البالغة المصابون بالسكري من النوع الثاني في مجموعة حبيبات العلف العادية (مجموعة A) و مجموعة حبيبات العلف عالية الدهون (مجموعة B) و مجموعة التغذية عالية الدهون (مجموعة اليراغلوتيد) (GLP-1 receptor agonist) الذي يضجح في الجهاز العصبي المركزي أو بشكل غير مباشر من اليراغلوتيد عن طريق فقان الوزن والتحكم في الجلوكوز عن طريق ارتفاع مستويات مصل الاديبوسايتوكينات، كهرباء غذائية عالية الدهون، التناول المزمن للفيتوكانثينات، وانخفاض إنتاج الأنسولين من الخلايا العصبية الطرفية.

استخدمت الدراسة الحالية ستة وثلاثون من ذكور جرذان ويرتدي بالبلاجة (وزن 200-220 جراماً)، و تم تقسيمهم إلى مجموعتين رئيسية: مجموعة حبيبات العلف العادية (مجموعة A) التي تضم 12 جرذًا، و تلتقي حبيبات التغذية من النوع الثاني، و مجموعة حبيبات العلف عالية الدهون (مجموعة B) والتي تضم 24 جرذًا، و تلتقي حبيبات التغذية عالية الدهون. ثم قسمت الدراسات إلى مستويات التغذية عايدة للدهون، و ثم تمّ إعطاءها 8 أسابيع للاستجابة بها. لم تتلون مجموعة ب، وانتظمت أعراض انخفاض السكري والعالية الدهون لمدة 8 أسابيع ثم تلتقي 600 ميكروغرام / كجم / يوم من اليراغلوتيد و 0.5 مل / كجم من المحلول المثلي الخاص بصفاق لمدة أربعة أسابيع بوضوح جيدة مصلية للالتهابات من النوع الثاني، و رمضان الخبيط.

المستندات المتميزة: مقاومة الأنسولين، حبيبات العلف عالية الدهون، اليراغلوتيد، الابيديوسايكتينيات.
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 [4]. 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 [5].

Adipose tissue 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 [6, 7]; 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 [8]. White adipose tissue 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 adipocytokines 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 [6, 7]. A wide range of biological activities including homeostatic and pathological function are regulated by these adipocytokines [9]. Obesity causing dysregulation of AT functions resulting in increased secretion of adipocytokines and pro-inflammatory cytokines like resistin, inducing insulin resistance and endothelial dysfunctions [10].

Adipose tissue form a cross-communication network between different organs in the body that reflects the diversity of the physiological role of adipocytokines [6]. 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 [11]. 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 cluster like structure (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 [12]. 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 adipose tissue macrophage (ATM) during chronic obesity is circulating blood monocytes [13]. 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 [14]. Adipokines act in paracrine, autocrine or endocrine manner to regulate various metabolic functions, adipokines act locally or systemically and implicated in regulation of insulin sensitivities in insulin-sensitive organs like liver [15]. Adipokines are classified into: pro-inflammatory and anti-inflammatory adipokines depending on the inflammatory response of AT [16]. Liraglutide improve meal-stimulated insulin secretion so it 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. Glucagon like peptide-1 receptor agonist 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 [17].

Materials and methods:
Materials:
Chemicals used in this study were with high quality and involve: ketamin (kepro Holland), xylazine (kepro Holland), liraglutide 1.8mg (Novo-Nordisk), normal saline (pioneer), As well as a specific sandwich ELISA kits from Mybiosource USA company for detection of serum adiponectin, leptin, omentin-1, insulin, visfatin and acylated ghrelin using microplate washer (BioTek ELx50) and reader (BioTek ELx800).

Experimental design:
The current study utilized thirty-six adult male wistar rats (weighing 200-220gm), they were divided into two main groups: normal diet group (group A) that includes 12 rats receiving normal pellets and high fat diet group (group B and C) which has 24 rats feeding high fat diet pellets. Animals fed high fat diet pellets for 8 weeks to induce insulin resistance were divided into two groups as seen in table-1.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Treatment</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (control)</td>
<td>12</td>
<td>Feeding normal diet pellets for 8 weeks then normal diet pellets + 0.5ml/kg normal saline intraperitoneal four weeks.</td>
<td>Twelve weeks</td>
</tr>
<tr>
<td>B (Insulin resistance)</td>
<td>12</td>
<td>Receiving HFD pellets for 8 weeks then administered 0.5ml/kg normal saline intraperitoneal for four weeks plus high fat diet pellets.</td>
<td>Twelve weeks</td>
</tr>
<tr>
<td>C (treatment)</td>
<td>12</td>
<td>Receiving HFD pellets for 8 weeks then received 600μg/kg/day intraperitoneal liraglutide +0.5ml/kg normal saline four weeks along with high fat diet pellets.</td>
<td>Twelve weeks</td>
</tr>
</tbody>
</table>
Liraglutide (victoza\textsuperscript{®}) was given intraperitoneally (600μg/kg daily dose) freshly prepared in normal saline 0.5ml/kg for four weeks, treatment was started at the end of the 8\textsuperscript{th} week of chowing HFD\textsuperscript{[18, 19]}. At the end of the 12\textsuperscript{th} week, all rats were fasted for 3 hours then anesthetized by administration of 5 mg/kg xylazine and 50 mg/kg ketamine intramuscularly\textsuperscript{[20]}, blood collected by syringe from animals (~8 ml) via cardiac puncture and the anesthetized animals euthanized by decapitation\textsuperscript{[21]}.

**Methods:**
The obtained 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\degree\textsuperscript{C}, take out the serum and put it in Eppendorf tubes, samples were stored in deep freezer at (-20 °C or -80 °C) for detection of insulin, leptin, visfatin, acylated ghrelin, adiponectin, and omentin-1 levels; evade freeze / thaw cycles because it is harmful.

**Statistical analysis:**
The statistical packages for social sciences (SPSS version 16) was used for statistical analysis \textsuperscript{[22]}. Descriptive data analysis represented as mean ± standard error of mean (SEM), One-Way ANOVA test used to discriminate between more than two independent means of all studied biomarkers of the three groups, pursued by Tukey test. Paired T-test was used for comparison between means of initial and final blood glucose and body weight of the same group\textsuperscript{[23]}. Statistically significant change was considered at p ≤ 0.05.

**Results:**
Statistical data analysis of all studied parameters was expressed as mean ±SEM and revealed that feeding high fat diet pellets produce a significant * increase in serum insulin, leptin and visfatin as well as significant decrease in serum level of adiponectin, omentin-1 and acylated ghrelin of HFD group when compared with control group. While, liraglutide produce a significant* reduction in serum level of insulin, leptin and visfatin as well as significant* increase in serum level of adiponectin, omentin-1 and acylated ghrelin of HFD group when compared with control group as seen respectively figure (2 and 3) A, B and C.
Figure-1 A: Serum insulin concentration, B: serum leptin concentration, C: serum visfatin concentration in ng/ml of all studied group, data expressed as mean ±SEM. * mean significant difference of group B when compared with group A or C; p≤ 0.05 mean significant difference between the groups.
Discussion: 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 \[24\]. While significant drop of serum insulin level of group C after daily administration of 600µg/kg liraglutide when compared with serum insulin level group B explained the effect of liraglutide on improving insulin sensitivity through activation of AMPK \[25\].

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 \[26\], 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 [27]. 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. Statistical analysis of this study revealed a markedly increase in serum visfatin level of group B that fed HFD for twelve weeks when compared with control group and this agreed with previous studies of HFD induce obesity associated with AT inflammation and insulin resistance that leading to increase serum level of pro-inflammatory adipokines like visfatin [28]. Serum visfatin level negatively regulated when body weight lost via daily administration of liraglutide to group C which give a significant decrease in serum visfatin level when compared with group B that receive HFD. This work consists with the anti-inflammatory effect of liraglutide that reduce visfatin level [29].

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) [30]. 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 [31]. 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 liraglutide for four weeks when compared with group B. 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 metabolic syndrome [32]. The present study reveals that HFD feeding markedly 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 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 [33]. Serum omentin-1 concentration inversely related to obesity-associated with insulin resistance and MS [34]. This completely consistent with the study of HFD effect on serum omentin-1 level of group B which significantly drop due to increased body weight and AT inflammation when compared with group A. Liraglutide by its anti-inflammatory effect and insulin sensitizing properties significantly increase serum omentin-1 level of group C after daily administration of liraglutide for four weeks when compared with group B that receive HFD pellets for twelve weeks in this study.

Conclusions:
According to the results of this study, one can conclude that:
• High fat diet pellets significantly increase body weight and induce insulin resistance.
• Liraglutide significantly lower blood glucose and reduce body weight.
• Liraglutide markedly elevates serum level of anti-inflammatory adipokines (adiponectin and omentin-1) and acylated ghrelin.
Liraglutide significantly lower serum level of insulin and pro-inflammatory adipocytokines (leptin and visfatin).

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